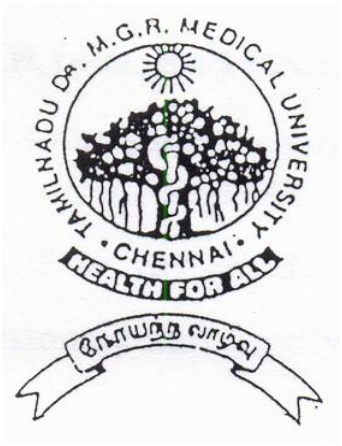
A STUDY OF SERUM FETUIN-A LEVEL IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Dissertation Submitted for

M.D DEGREE BRANCH - XIII

[BIOCHEMISTRY]



DEPARTMENT OF BIOCHEMISTRY

THANJAVUR MEDICAL COLLEGE,

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THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY,

CHENNAI

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CERTIFICATE

This is to certify that the dissertation titled "**A STUDY OF SERUM FETUIN-A LEVEL IN PATIENTS WITH CHRONIC KIDNEY DISEASE"** is a bonafide work done by **Dr.P.DEEPA,** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her postgraduate course from 2010 to 2013.

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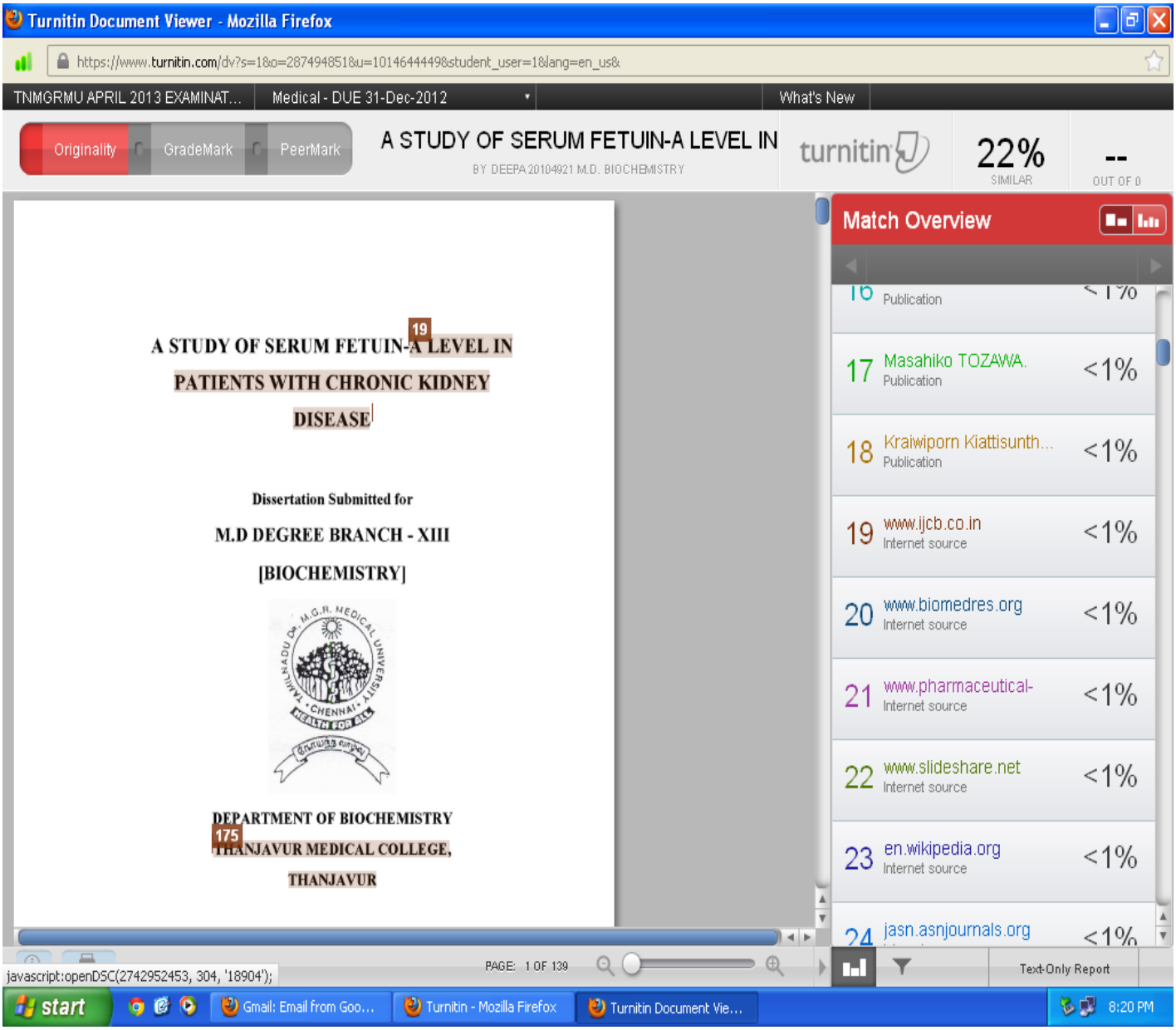
DECLARATION

I, **Dr.P.DEEPA**, hereby solemnly declare that the dissertation titled "**A STUDY OF SERUM FETUIN-A LEVEL IN PATIENTS WITH CHRONIC KIDNEY DISEASE"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the supervision and guidance of my Professor and Head of the Department **Dr.N.Sasivathanam, M.D(Bio).,DGO,**. This dissertation is submitted to the TamilNadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch – XIII) in Biochemistry.

Place : THANJAVUR (Dr.P.DEEPA)

Date :

ANTIPLAGIARISM – ORIGINALITY REPORT



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CONTENTS

ABBREVIATIONS

CKD	–	Chronic Kidney Disease
GFR	–	Glomerular Filtration Rate
K/DOQI	–	Kidney Disease Outcomes Quality Initiative
HDL-C	–	High Density Lipoprotein Cholesterol
VLDL-C	–	Very Low Density Lipoprotein Cholesterol
LDL-C	–	Low Density Lipoprotein Cholesterol
TGL	–	Triglycerides
TC	–	Total Cholesterol
hsCRP	–	High sensitivity C-Reactive Protein
CKD-MBD	–	Chronic Kidney Disease-Mineral-Bone Disorder
TGF-β	–	Transforming Growth Factor-β
BCP	–	Basic Calcium Phosphate
Ca X P	–	Calcium-Phosphorus product
C_{cr}	–	Creatinine clearance
FBG	–	Fasting Blood Glucose

A STUDY OF SERUM FETUIN-A LEVEL IN PATIENTS WITH CHRONIC KIDNEY DISEASE

ABSTRACT

BACKGROUND AND OBJECTIVES:

Chronic Kidney Disease (CKD) is characterized by excessive vascular calcification which in turn is associated with cardiovascular dysfunction. Inflammation and derangements in calcium-phosphorus metabolism seem to play an important role in the accelerated calcifying atherosclerosis seen in CKD. Fetuin-A is the prototype of a systemically acting inhibitor of extraskeletal calcification and is downregulated following inflammation. In the present study, serum Fetuin-A level was estimated in patients with CKD and its relationship with inflammation and alteration in calcium-phosphorus levels were analyzed.

MATERIALS AND METHODS:

80 patients with CKD and 80 healthy controls were enrolled in the study. Serum levels of Fetuin-A, hsCRP, calcium, phosphorus, albumin and lipid profile were measured. Serum Fetuin-A was estimated by Enzyme Linked Immunosorbent Assay (ELISA).

RESULTS:

Compared to controls, a significant reduction of serum Fetuin-A level was observed in patients with CKD and this reduction was gradual,

beginning from the early stages of CKD. Serum Fetuin-A levels also showed a significant negative correlation with hsCRP and calcium-phosphorus levels and a significant positive correlation with albumin levels.

CONCLUSION:

The results of the present study indicate that in CKD, there is a progressive reduction in serum Fetuin-A levels along with the gradual decline in renal function. The chronic inflammatory state and the altered mineral metabolism prevailing in CKD could be responsible for the lowered levels of serum Fetuin-A.

KEY WORDS:

Chronic Kidney Disease, Fetuin-A, hsCRP, Inflammation

INTRODUCTION

Chronic kidney disease (CKD) refers to an irreversible progressive deterioration in renal function[1]. CKD is defined as either kidney damage or GFR < 60 ml/min/1.73 m^2 for 3 months or more, irrespective of the cause. In CKD, there is a progressive and unrelenting loss of renal function which ultimately leads on to end stage renal disease[2].

CKD is a worldwide, chronic, non-communicable disease epidemic with adverse outcomes of renal failure, cardiovascular disease and premature death. In developed countries, it affects 10-15% of adult general population. Prevalence of CKD in India is 0.79%[21]. Diabetes mellitus and hypertension are responsible for 40-50% of all cases of CKD.

Accelerated cardiovascular disease is a frequent complication of CKD. Over 80-90% of patients with CKD die primarily of cardiovascular disease, before reaching the need for dialysis[3]. This emphasizes the importance of early detection of cardiovascular disease, before the patients reach advanced stages of CKD.

Alteration of mineral metabolism that occurs in CKD promotes vascular calcification. In addition, vascular calcification also involves the imbalance of equilibrium between calcification promoters and inhibitors. The presence of vascular calcification poses an increased risk of cardiovascular and all-cause mortality in patients with CKD[4].

CKD is also associated with chronic inflammation, which promotes endothelial dysfunction, vascular remodelling and progression of atherosclerosis. In CKD, progressive deterioration of renal function may also lead on to accumulation of uremic toxins and dyslipidemia, which in turn stimulate inflammation and result in atherosclerosis[5].

Hence in CKD, there exists an active interplay between atherosclerosis and vascular calcification through inflammation, against a background of severe calcium-phosphorus disturbances. This in turn contributes to the development of cardiovascular disease in patients with CKD.

Serum Fetuin-A, an α_2-glycoprotein is regarded as a negative acute phase reactant, that is down regulated following inflammation[6]. Further, Fetuin-A has also been identified as a potent circulating inhibitor of systemic calcification by inhibition of calcium-phosphate precipitation[7]. Measurement of serum Fetuin-A levels could therefore have a potential value to predict vascular calcification and hence the cardiovascular risk in CKD.

Hence in the present study, the serum level of Fetuin-A was estimated in patients with CKD and the relationship between serum Fetuin-A, inflammation, abnormalities in calcium-phosphorus levels and dyslipidemia were analyzed.

REVIEW OF LITERATURE

The kidneys excrete metabolic waste products and have an essential homeostatic function by controlling the body solute and water status and the acid-base balance. In addition, the kidneys have important endocrine functions including production of calcitriol, erythropoietin and renin[8].

RENAL EXCRETORY MECHANISMS – AN OVERVIEW:

RENAL GLOMERULAR FUNCTION:

About 200L of plasma ultra filtrate enter the renal tubular lamina daily by glomerular filtration[9]. Glomerular filtration rate depends on the following factors:

(i) Balance of pressures across the filtration barrier in the glomerulus (difference between the hydrostatic pressure in the glomerular capillaries which promote filtration, and the plasma oncotic pressure and hydrostatic pressure in Bowman's space which oppose filtration)

(ii) Rate of renal blood flow

(iii) Total surface area of the glomerular capillaries

GLOMERULAR FILTRATION BARRIER:

An ultra filtrate of plasma passes from glomerular capillary blood into the space of Bowman's capsule, through the glomerular filtration barrier[10,11].

The glomerular filtration barrier is made up of 3 layers:

1. Capillary endothelium
2. Basement membrane
3. Podocytes

Glomerular endothelial cells and podocytes have glycocalyx, a negatively charged surface coat. The glomerular basement membrane also contains negatively charged heparan sulphate, sialic acid and sialoproteins. These negative charges impede the passage of negatively charged molecules through the glomerular filtration barrier by electrostatic repulsion.

RENAL TUBULAR FUNCTION:[8,12,13]

When the plasma filtered into Bowman's space enters the proximal tubule, the process of reabsorption takes place. From the 200L of plasma filtered daily, only about 2L of urine are formed. Almost all the reusable nutrients and the bulk of electrolytes are reclaimed from the proximal tubules, with fine homeostatic adjustments taking place more distally.

The tubular cells do not actively deal with waste products like urea and creatinine to any significant degree. Most filtered urea is passed in urine, but some amount of it diffuses back passively from the collecting ducts with water; by contrast, some amount of creatinine is secreted into the tubular lumen.

The tubular cells use adenosine tri-phosphate dependent active transport, sometimes selectively, against physicochemical gradients. Transport of charged ions tends to produce an electrochemical gradient that inhibits further transport. This is minimized by 2 processes:

(i) ISOSMOTIC TRANSPORT:

This occurs mainly in the proximal convulted tubules and reclaims the bulk of filtered essential constituents. Active transport of one ion leads to the passive movement of another ion of the opposite charge in the same direction along the electrochemical gradient. For example, isosmotic reabsorption of Na^+ depends on the availability of negatively charged ions like Cl^-. The process is "isosmotic" because the active transport of solute causes movement of equivalent amount of water in the same direction. Isosmotic transport also occurs to a lesser extent in the distal parts of the nephron.

(i) ION EXCHANGE:

This occurs mainly in the more distal parts of the nephrons and is important for fine adjustment after bulk reabsorption has taken place. Ions of the same charge, usually cations are exchanged and neither electrochemical nor osmotic gradients are created. For example, Na^+ may be reabsorbed in exchange for K^+ and H^+ ions.

REQUIREMENTS FOR NORMAL RENAL FUNCTION:

The function of the two kidneys reflects the sum of the functions of their individual nephrons. For a nephron to function normally, the following conditions must be satisfied:[14]

1. There must be free flow of blood through the glomerular capillaries.
2. The glomerular filter must function normally. An adequate volume of filtrate must be produced, but the filter must restrict the passage of blood cells and proteins.
3. The tubules must be able to selectively reabsorb important substances from the filtrate and to excrete other constituents into the filtrate.
4. The urine formed by the nephron must be able to flow freely from the kidney into the bladder and out of the urethra.

Derangement of any of these functions results in kidney disease.

CHRONIC KIDNEY DISEASE

Chronic kidney disease is a syndrome of persistent renal impairment involving the loss of both glomerular and tubular functions, such that the homeostatic functions of kidneys are compromised.

In 2002, the United States National Kidney Foundation published the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines, which provided a framework for the evaluation of CKD.

DEFINITION OF CKD:[15]

CKD is defined as "either kidney damage for ≥ 3 months or a glomerular filtration rate < 60 ml/min/1.73 m^2 for ≥ 3 months irrespective of the cause". Kidney damage is defined by structural or functional abnormalities of the kidney with or without decreased glomerular filtration rate, manifesting by either,

- Pathological abnormalities, (or)
- Markers of kidney damage, including abnormalities in the composition of blood or urine, (or)
- Abnormalities in imaging tests.

STAGES OF CKD:[16]

The National Kidney Foundation-K/DOQI classification system for stages of CKD is based on the severity of the disease as indicated by the

level of glomerular filtration rate. The higher stages represent lower GFR levels, regardless of the rate of progression or the specific cause.

CKD STAGE	DESCRIPTION	GFR (ml/min/1.73m^2)
0	Individuals at increased risk	≥ 90
1	Kidney damage with normal or increased GFR	≥ 90
2	Kidney damage with mild decrease in GFR	60-89
3	Moderate decrease in GFR	30-59
4	Severe decrease in GFR	15-29
5	Kidney failure	< 15

GFR can be assessed by either 24 hours urinary creatinine clearance or from serum creatinine by using one of the following formulas:[17,18]

- Cock-Croft Gault formula
- Modification of Diet in Renal Disease (MDRD) formula
- CKD-Epidemiology Collaboration (CKD-EPI) equation

EPIDEMIOLOGY:

CKD is a worldwide threat to public health, but the true incidence and prevalence of CKD within a community are difficult to ascertain because, early to moderate CKD is usually asymptomatic[19]. CKD has remained largely as a 'silent' epidemic.

According to World Health Organisation Global Burden of Disease Project, kidney and urinary tract diseases contribute to the global burden with approximately 8,50,000 deaths every year. Globally, CKD has been found to be the 17th cause of disability and 12th leading cause of death[20].

In India, the prevalence of CKD is 0.79% and 10% of patients with CKD are in the end stage renal disease[21,22].

NATURAL HISTORY OF CKD:

It has been predicted that about two-thirds of normal adults, above the age of 40 years will experience a decrease in GFR, even though they might not have had any obvious kidney disease in the past, whereas in the remaining one-third of adults, GFR has been shown to remain stable. However, this decrease of GFR with age differs from the progressive deterioration of renal function that follows kidney damage and rarely requires therapy[23].

Most patients with CKD stages 3-5, progress relentlessly to end stage renal disease. The rate of progression of CKD depends on the following factors:[24]

- varies according to the underlying nephropathy
- varies between individual patients
- quality of control of glycemia and systemic hypertension
- secondary to infections, dehydration or exposure to nephrotoxins like non-steroidal anti-inflammatory drugs or radiocontrast agents

The rate of decline of GFR is fastest in patients with diabetic nephropathy, averaging ~ 10 ml/min/year. Rate of decline is also faster in polycystic kidney disease and chronic glomerulonephritis.

RISK FACTORS OF CKD:[25,26]

There are various factors which affect initiation and progression of CKD.

(i) Susceptibility factors

- Increase the susceptibility to kidney damage

(ii) Initiation factors

- Directly initiate the kidney damage

(iii) Progression factors

- Cause worsening of kidney damage and faster decline in kidney function

(iv) End stage factors

- Increase the morbidity and mortality in kidney failure

TYPES OF RISK FACTORS FOR CKD:

RISK FACTORS	EXAMPLES
Susceptibility factors	Older age, family history, racial or ethnic minority status, low birth weight, low income
Initiation factors	Diabetes, hypertension, urinary tract infection, lower urinary tract obstruction, drug toxicity like non-steroidal anti-inflammmatory drugs, autoimmune diseases, systemic infections
Progression factors	Poor glycemic control in diabetes, uncontrolled hyper tension, smoking, higher levels of proteinuria
End stage factors	Low serum albumin, anaemia, temporary vascular access, lower dialysis dose (K_t/V)

AETIOLOGY OF CHRONIC KIDNEY DISEASE:[27,28]

(i) Congenital and inherited diseases:

- Polycystic kidney disease
- Medullary cystic disease
- Alport's syndrome
- Tuberous sclerosis
- Oxalosis
- Congenital obstructive uropathy
- Cystinosis

(ii) Glomerular diseases:

- Primary glomerulonephritis including focal glomerulosclerosis

(iii) Secondary glomerular disease (Systemic Lupus Erythematosus, Polyangiitis, Wegener's Granulomatosis, Haemolytic Uremic Syndrome, Thrombotic Thrombocytopenic Purpura, Amyloidosis, Diabetic Glomerulosclerosis, accelerated Hypertension, Systemic Sclerosis, Henosch-Schonlein Purpura, Sickle Cell Disease)

(iv) Vascular diseases:

- Hypertensive nephrosclerosis
- Small and medium-sized vessel vasculitis
- Renovascular disease

(v) Tubulointerstitial diseases:

- Tubulointerstitial nephritis - idiopathic, due to drugs (especially nephrotoxic analgesics), immunologically mediated
- Schistosomiasis
- Nephrocalcinosis
- Reflux nephropathy
- Balkan nephropathy
- Tuberculosis
- Myeloma kidney
- Renal papillary necrosis (Diabetes, Sickle Cell Disease and Trait, Analgesic nephropathy)

(vi) Urinary tract obstruction:

- Calculus disease
- Pelvic tumors
- Retroperitoneal fibrosis
- Prostatic disease
- Schistosomiasis

PATHOPHYSIOLOGY OF CKD:

2 broad sets of mechanisms of damage are involved in the pathophysiology of CKD:[29]

(i) Initiating mechanisms that are specific to the underlying etiology like genetically determined abnormalities in kidney development or toxin exposure or immune complex deposition and inflammation.

(ii) Progressive mechanisms that involve hyperfiltration and hypertrophy of the remaining viable nephrons.

The pathophysiologic progression of CKD is characterized by,

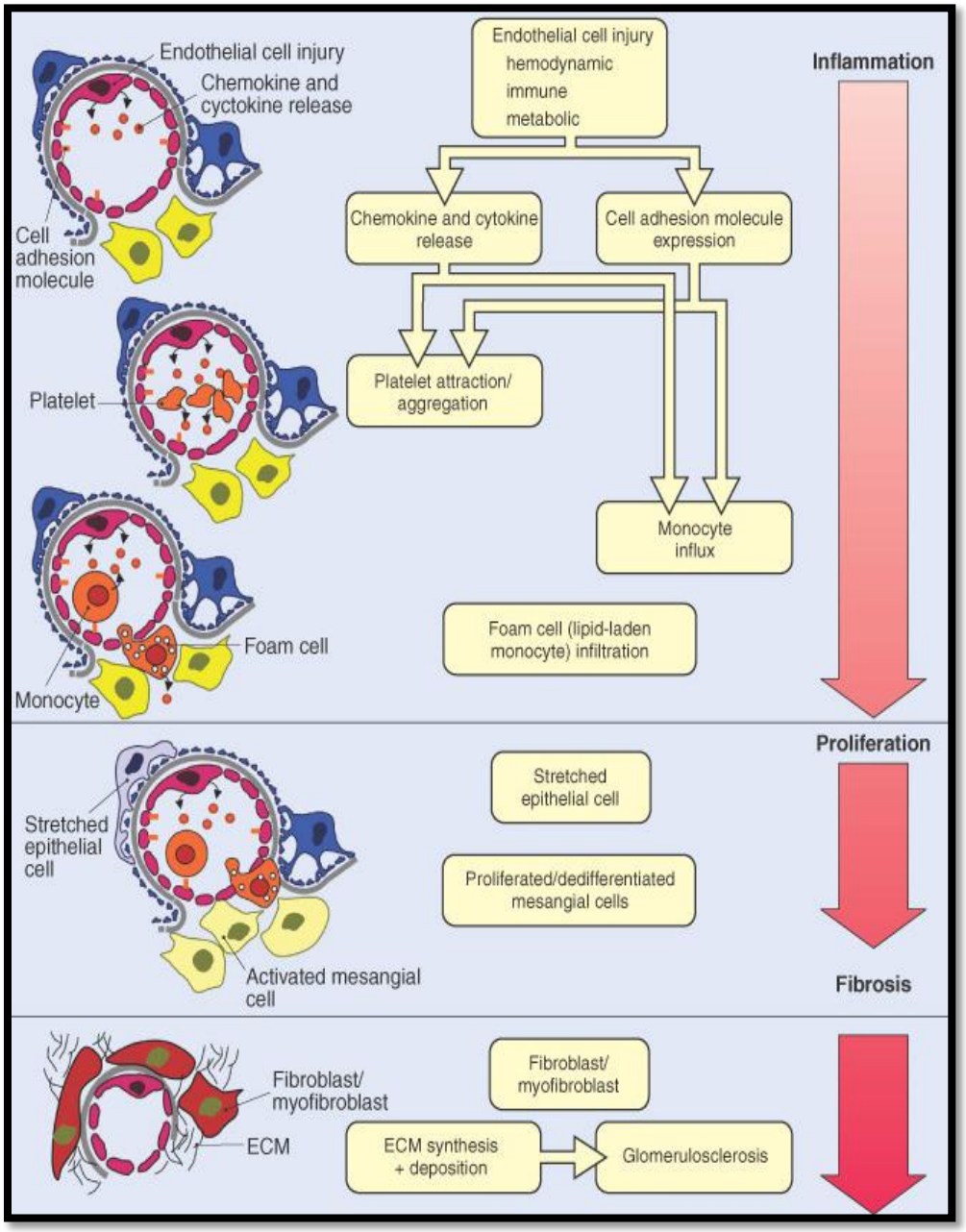
• Glomerulosclerosis and

• Tubulointerstitial inflammation and fibrosis

GLOMERULOSCLEROSIS:[30]

This is characterized by 3 stages. Figure 1 shows the stages of glomerulosclerosis.

Figure 1: STAGES OF GLOMERULOSCLEROSIS



● Stage of inflammation:

- Within the glomerular capillaries, endothelial cells are the first to be exposed to damage induced by hemodynamic, immunologic or metabolic insults.
- The glomerular endothelial injury is associated with reduction or loss of their physiologic anticoagulant and anti-inflammatory properties and acquisition of pro-coagulant and inflammatory characteristics.
- This leads to attraction and activation of platelets and micro thrombus formation in glomerular capillaries.
- These changes lead to the initiation of glomerular micro inflammation, with the attraction, adhesion and infiltration of glomerular tufts by inflammatory cells.

● Stage of proliferation:

After endothelial injury and the ensuing micro inflammatory response, infiltrating monocytes interact with mesangial cells and stimulate their proliferation either through cell-cell interactions or through the release of mitogens.

● Stage of fibrosis:

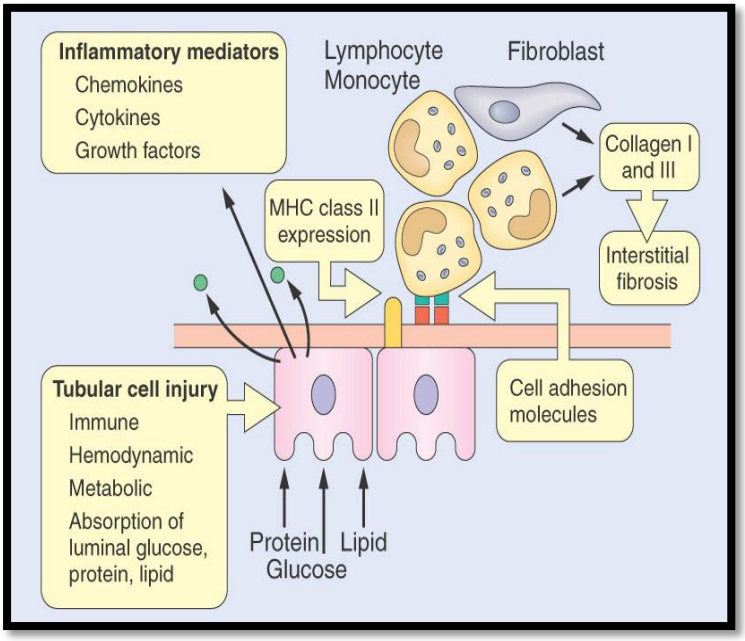
- Under the control of fibrogenic growth factors like TGF-β1, proliferating and activated mesangial cells have the capacity to revert to a mesenchymal phenotype (myofibroblasts) which synthesize a range of extracellular matrix components.

- The relative inability of the podocytes to replicate in response to injury may lead to their stretching along the glomerular basement membrane, thereby exposing its denuded areas, which in turn interact with parietal epithelial cells and form capsular adhesions.
- The subsequent disruption of glomerular-tubular junction, result in atubular glomeruli. This may lead to misdirected filtration and accumulation of amorphous material in the paraglomerular space.

TUBULOINTERSTITIAL INFLAMMATION AND FIBROSIS:[31]

Like glomerulosclerosis, tubulointerstitial fibrosis also evolves in stages including inflammation, proliferation of interstitial fibroblasts, epithelial-mesenchymal transition and excessive deposition of interstitial extra cellular matrix (Figure 2).

Figure 2: TUBULOINTERSTITIAL FIBROSIS



MECHANISMS:

- Loss of renal mass leads to hyperfiltration of remaining nephrons which produces hypertension locally in the capillary tufts. This results in leakage of proteins into the tubular fluid.
- Injury to the glomerulus also results in addition of activated mediators to the proteinuric filtrate.
- As albumin is abundant in the filtrate, it binds to the activated mediators. These small albumin-bound molecules initiate the tubular inflammation.
- These complex mixtures of proteinuric cytokines bathe the tubular epithelia which produce increased amounts of chemokines, fibroblast growth factor-2, TGF-β and platelet derived growth factor.
- Proteinuric infiltrate also causes direct tubulo-interstitial injury by producing accumulation of proteins in the interstitial space and thereby activating the mediators that promote inflammation and fibrosis.
- AngiotensinII which promotes glomerular hyperfiltration and hypertension also contributes to proteinuria. It may also increase the activity of growth factors and inflammatory cells that mediate tubulointerstitial fibrosis and scarring.
- Also glomerular injury may reduce the peritubular perfusion and initiate local ischemia and hypoxia causing tubular atrophy and interstitial fibrosis.

COMPLICATIONS OF CKD:[32]

As the renal function declines progressively, the kidney loses its regulatory capacity which results in various complications:

(i) Fluid and electrolyte disturbances:

Increased or reduced blood volume, low or high sodium levels, hyperkalemia, hyperphosphatemia, hypocalcemia, metabolic acidosis.

(ii) Neurological abnormalities:

Headache, irritability and sleep disorders, seizures, peripheral neuropathy, muscle twitchings, cramps, coma.

(iii) Cardiovascular system:

Hypertension, heart failure, cardiomyopathy, pericarditis

(iv) Gastrointestinal tract:

Nausea, vomiting, anorexia, gastrointestinal bleeding, gastritis, uremic fetor

(v) Haematological abnormalities:

Anaemia, bleeding, coagulation defect, infections

(vi) Skin:

Pruritus, pallor, hyperpigmentation, ecchymoses, uremic frost

(vii) Metabolic and endocrinal abnormalities:

Glucose intolerance, secondary hyperparathyroidism, goitre, amenorrhoea, renal osteodystrophy

COMPLICATIONS IN VARIOUS STAGES OF CKD:

STAGES 1 & 2:

- Patient is asymptomatic.
- Blood urea nitrogen and serum creatinine are normal or near normal.
- Acid-base, fluid and electrolyte balances are maintained through an adaptive increase of function in the remaining nephrons.

STAGE 3:

- Serum creatinine and blood urea nitrogen are elevated.
- Serum levels of erythropoietin, calcitriol and paratharmone are usually abnormal.
- Patients present with anaemia, hypertension, fluid retention and dyslipidemia.

STAGE 4:

- This stage is characterized by severe impairment of GFR involving a further loss of kidney function.
- Along with features of stage 3, patients present with malnutrition,acidosis, hyperphosphatemia, hypocalcemia, hyperkalemia, amenorrhoea and infertility.

STAGE 5:

This stage is defined by a GFR < 15 ml/min/1.73 m^2 and is characterized by worsening of all the aforementioned findings along with bleeding diathesis, uremic encephalopathy, pericarditis and symptoms like fatigue, dysgeusia, anorexia, nausea and pruritus.

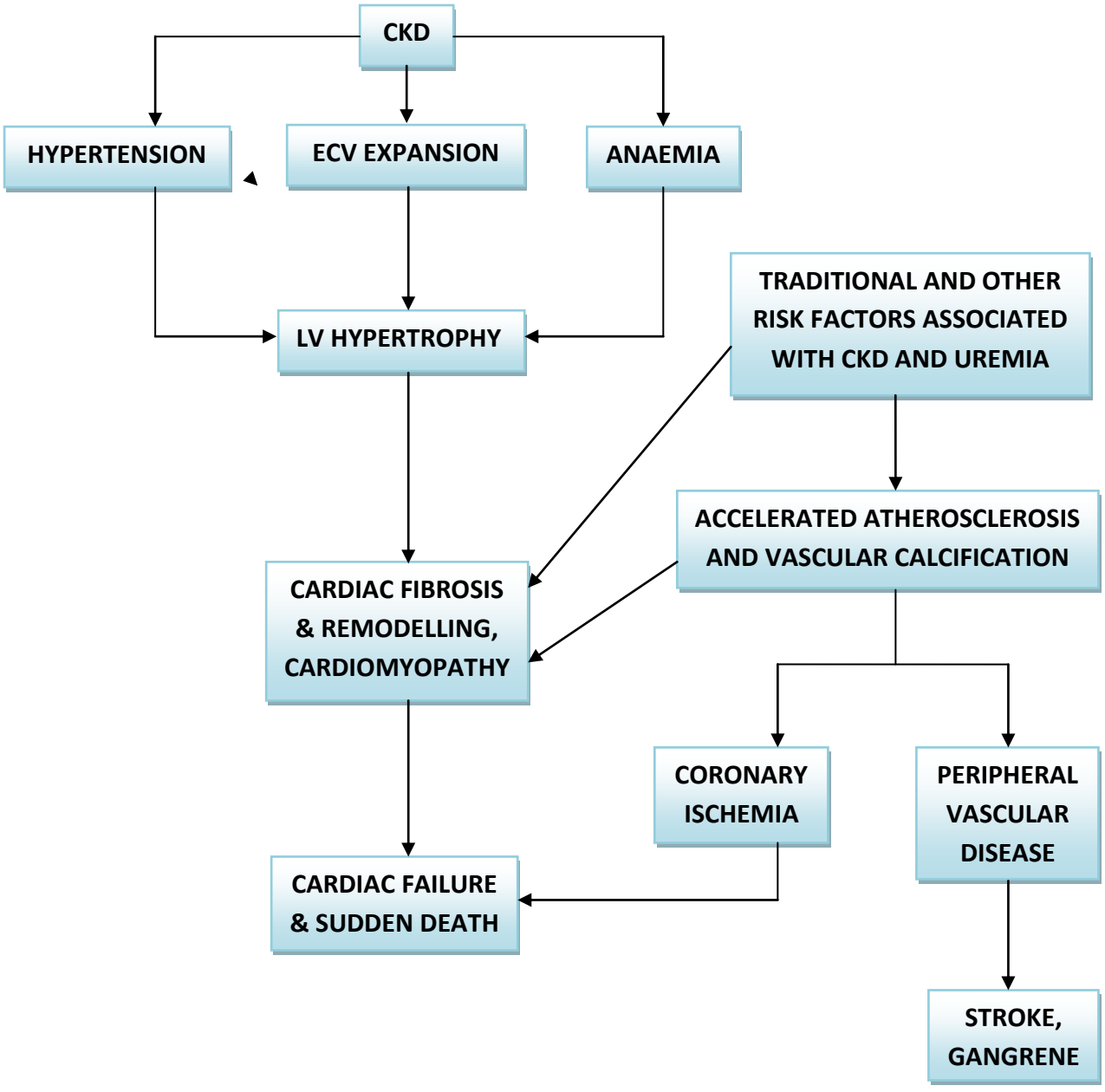
CARDIOVASCULAR DISEASE IN CHRONIC KIDNEY DISEASE:

Cardiovascular disease is the most frequent cause of death among people with CKD. The overall mortality rate from cardiovascular disease in CKD patients has been found to be about 30 times greater than that of general population[33]. CKD is therefore considered as a "cardiovascular risk equivalent".

In patients with CKD, cardiovascular disease is characterized by left ventricular hypertrophy, which results largely due to expansion of extracellular volume, anaemia and hypertension. Left ventricular remodelling and fibrosis may accompany left ventricular hypertrophy which ultimately results in several complications[34]. Figure 3 shows the mechanisms involved in the development of cardiovascular disease in CKD.

Cardiovascular complications associated with CKD include myocardial infarction, angina pectoris, arrhythmias, cardiac failure, peripheral vascular disease, stroke and sudden death. The risk increases from early stages to advanced CKD for each of these conditions.

Fig.3: CARDIOVASCULAR DISEASE IN PATIENTS WITH CKD



CARDIOVASCULAR RISK FACTORS IN CKD:[35,36]

TRADITIONAL RISK FACTORS:

- Age, Gender, Diabetes, Insulin Resistance, Hypertension, Smoking

NON-TRADITIONAL/NOVEL RISK FACTORS:

- Inflammation
- Oxidative stress
- Endothelial dysfunction
- Anaemia
- Hyperphosphatemia
- Secondary hyperparathyroidism
- Vascular calcification
- Advanced Glycation End Products
- Hyperhomocysteinemia

DYSLIPIDEMIA IN CKD:[37,38]

Progressive deterioration of renal function results in altered composition of blood lipids which in turn predisposes to the development of vascular disease. Renal dyslipidemia is characterized by the following features:

- Hepatic apo-AI synthesis is decreased and lecithin cholesterol acyl transferase activity is reduced. This leads to decreased HDL-C levels.
- Increased synthesis of apo-CIII, a competitive inhibitor of lipoprotein lipase, leads to elevated levels of VLDL-C and chylomicrons, which

results in hypertriglyceridemia. Further, uremic toxins and secondary hyperparathyroidism reduces the levels of lipoprotein lipase which results in impaired catabolism of triglyceride rich lipoproteins. Insulin resistance associated with CKD also increases the VLDL-C levels.

- Total and LDL-cholesterol levels are usually normal, but may be low in patients with concomitant inflammation and malnutrition. There is characteristic accumulation of small dense atherogenic LDL-C.
- As GFR declines, the levels of high molecular weight isoforms of lipoprotein(a) increase, which is associated with increased cardio vascular risk.
- The changes in lipoprotein composition and structure in CKD stimulate and amplify the already existing inflammatory mechanisms, which in turn results in endothelial dysfunction and atherosclerotic progression[39,40].

INFLAMMATION IN CKD:[41,42,43]

Most CKD patients are in a state of chronic inflammation. Various factors may be associated with a sustained inflammatory response in CKD which include,

- Genetic background
- Persistence of inflammatory conditions

  - Exogenous (Foreign, example: bacteria, viruses etc…)

  - Endogenous (Reactive oxygen species, glycated and oxidized adducts,

renin-angiotensin activating system)

- Failure of clearance of inflammatory mediators

  - Retained solutes (cytokines and other mediators and glycated, oxidized adducts)

- Dysmetabolic states

  - Dyslipidemia

  - Central (visceral) obesity

  - Insulin resistance

In the clinical setting, the most commonly used biomarker of inflammation is C-Reactive Protein (CRP), one of the member of pentraxin family and the prototypic acute phase reactant. Other acute phase reactants include serum amyloid A, ferritin and fibrinogen. These proteins are the forward or positive acute phase reactants, whose serum levels increase during inflammation. The reverse or the negative acute phase reactants include albumin, prealbumin (transthyretin) and Fetuin-A and the level of these proteins fall during inflammation.

CRP, the prototypic inflammatory marker, inter leukin-6, and fibrinogen are the independent predictors of mortality in CKD patients. These markers have been attributed to their pro-atherogenic properties such as promotion of vascular calcification, oxidative stress and endothelial dysfunction.

VASCULAR CALCIFICATION IN CKD:

DISTURBANCES IN CALCIUM-PHOSPHORUS HOMEOSTASIS IN CKD:[44]

- Reduced GFR in CKD patients results in decreased excretion of phosphate, leading to severe hyperphosphatemia.
- Impaired vitamin D synthesis in the body leads to hypocalcemia which is the trigger for excessive secretion of paratharmone leading onto secondary hyperparathyroidism.

The disturbances in calcium-phosphorus homeostasis have been found to play a vital role in the pathogenesis of vascular calcification in CKD.

TYPES OF VASCULAR CALCIFICATION:[45]

(I) Atherosclerotic/intimal calcification:

- Occurs in the intimal layer and associated with plaques and occlusive lesions

(II) Monkeberg sclerosis/medial calcification:

- Involves circumferential deposition of amorphous mineral forms in the elastic lamellae of medial layer

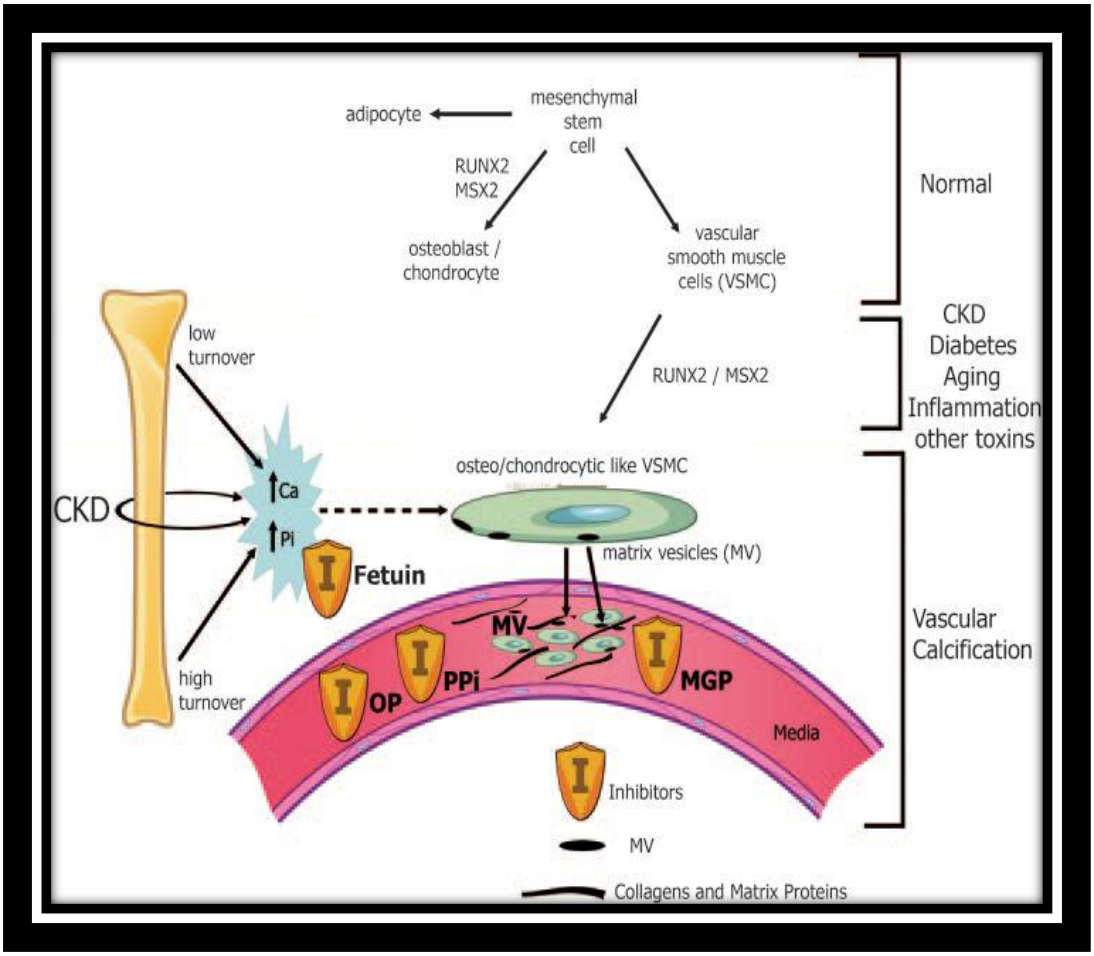
► Both types of vascular calcification are stimulated by CKD[46]. The hemodynamic consequences of vascular calcification like heart failure and

myocardial infarction are the major causes of mortality in most of the patients with CKD.

PATHOGENESIS:[47,48,49,50]

- Mesenchymal stem cells normally differentiate into chondrocytes, adipocytes, osteoblasts and vascular smooth muscle cells (VSMC).
- In the setting of CKD, there is upregulation of transcription factors like Cbfa1/RUNX-2 and MSX-2 that are critical for bone development in vascular smooth muscle cells. The upregulation of these transcription factors in VSMC is indicative of a phenotypic switch that results in dedifferentiation of these cells into osteo/ chondrocytic- like cells.
- This phenotypic transformation, lead onto the calcification of VSMC in a process similar to bone formation. These cells begin to lay down collagen and other noncollagenous proteins in the intima or media of the blood vessels (Figure 4).
- Further the extremes of bone turnover prevailing in CKD, alters the calcium and phosphorus content of the bone which results in increased blood levels of these minerals.
- Ultimately, whether an artery undergoes calcification or not depends on the strength of the army of circulating inhibitors like Fetuin-A and locally produced inhibitors like pyrophosphate and matrix-Gla protein.

Fig.4: MECHANISMS OF VASCULAR CALCIFICATION IN CKD



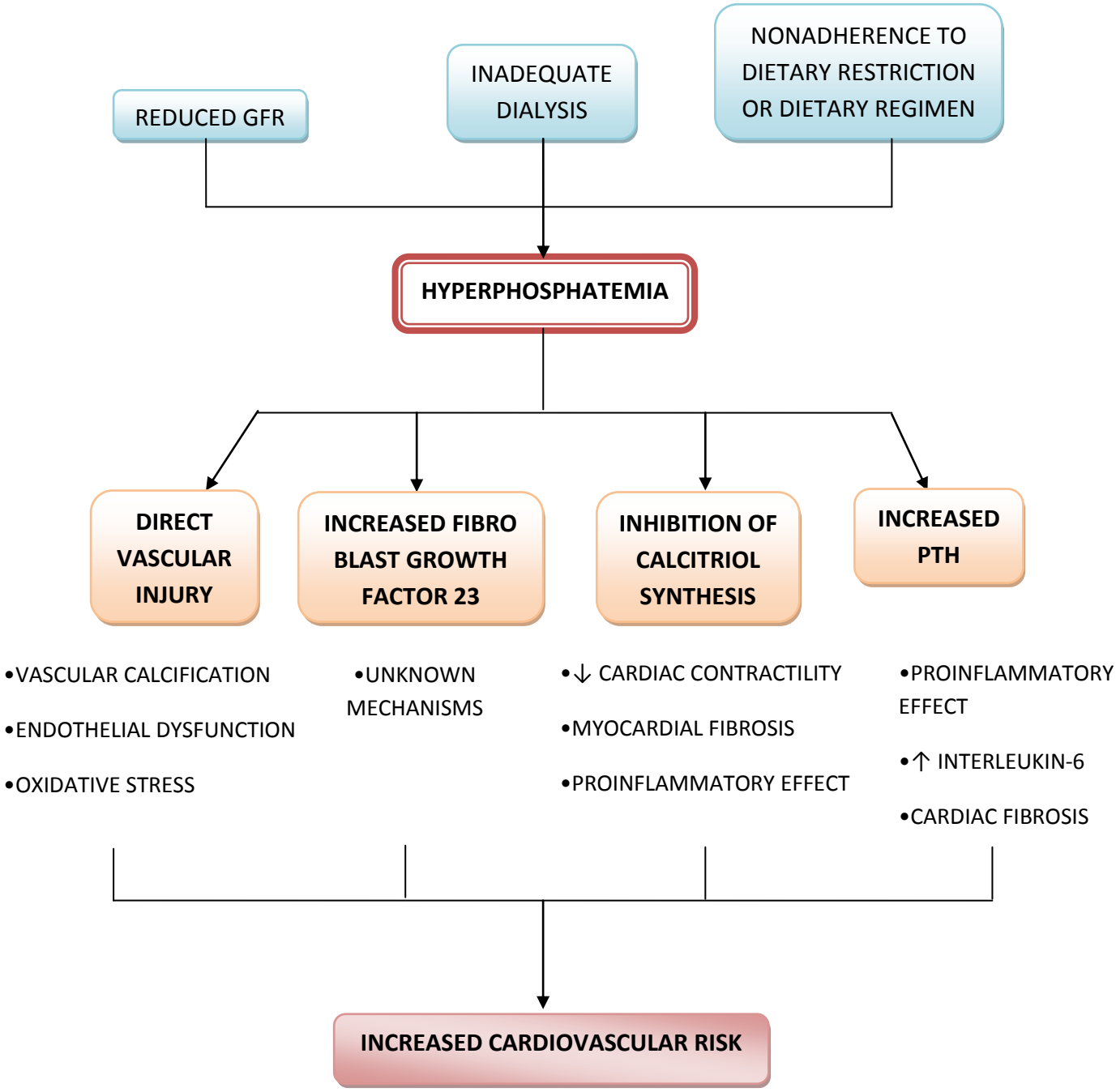
HYPERPHOSPHATEMIA IN CKD:

Elevated levels of serum phosphorus are associated with an increased risk of cardiovascular disease among patients with CKD. The ability of the kidneys to control serum phosphate levels becomes impaired at GFR ~50-60 ml/min[53].

The following mechanisms may be responsible for the pathogenic cardiovascular effects of hyperphosphatemia (Figure 5).

- Hyperphosphatemia directly causes vascular injury by promoting vascular calcification and increasing the reactive oxygen species.
- Indirectly, it increases the serum levels of paratharmone and fibroblast growth factor-23.
- Hyperphosphatemia may also promote or facilitate inflammation in patients with CKD. Since phosphorylation events regulate the important steps of inflammatory pathways, the increased serum phosphate levels may trigger phosphorylation-driven signaling inflammatory cascade[54,55].

Figure 5: HYPERPHOSPHATEMIA IN CKD



In appreciation of the wider consequences of disturbed mineral and bone metabolism in patients with CKD, a new nomenclature – "CKD-MBD" (mineral-bone disorder) has been introduced by the Kidney Disease Improving Global Outcomes (KDIGO), 2006 to describe this broader clinical syndrome.

DEFINITION OF CKD-MBD:[51,52]

A systemic disorder of mineral and bone metabolism due to CKD is manifested by either one or a combination of the following:

- Abnormalities of calcium, phosphorus, paratharmone and vitamin D metabolism
- Abnormalities in bone turn over, mineralization, volume, linear growth or strength
- Vascular or other soft tissue calcification

FETUIN-A / α_2–HEREMANS-SCHMID GLYCOPROTEIN (AHSG):

The serum protein Fetuin was initially identified as the major globulin in calf and fetal serum by Pedersen in 1944. The human homologue was named Fetuin-A/α_2–Heremans-Schmid glycoprotein (AHSG), after the two co-discoverers, Heremans and Schmid. A closely related protein, Fetuin-B also exists in human, rat and mouse genomes[56].

During fetal development, Fetuin-A is expressed in most organs including liver, kidney, gastrointestinal tract, skin and brain. In adults, it is produced primarily by the liver. The serum Fetuin-A concentration of adult humans ranges from 0.5-1 g/L[57,78].

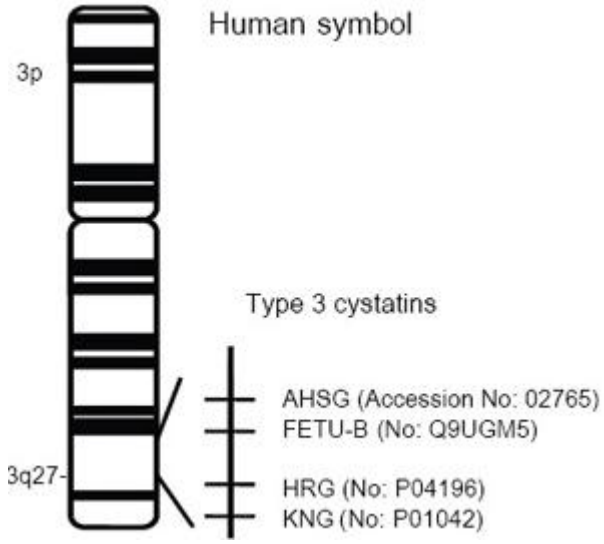
STRUCTURE:

Fetuin-A, a 59 kDa glycoprotein, belong to the cystatin superfamily of cysteine protease inhibitors. It contains 3 domains- D_1, D_2, D_3. D_1 and D_2 are cystatin like domains located in the amino terminal region. Domain 3 forms the C-terminal part of Fetuin-A and it is rich in proline (collagen like domain)[58].

Fetuin-A is composed of 2 subunits- the heavy ‘A’ chain and the light ‘B’ chain. ‘A’ chain consists of 282 aminoacid residues while ‘B’ chain consists of 27 residues. A disulfide bridge between cys-14 and cys-340 joins both the chains. There are also 5 intra chain disulfide bridges in the ‘A’ chain.

Figure 6: STRUCTURE OF FETUIN-A

Figure 7: FETUIN-A GENE



Fetuin-A has a binding site for calcium-phosphate near the N-terminus. A TGF-β cytokine binding motif is present from cys-96 to cys-114 in the N-terminal cystatin domain. This motif has sequences similar to that of the extracellular domain of TGF-β receptor type II [59].

The molecule also has interesting and unique features:[60]

- In circulating Fetuin-A, there are 29 aminoacid doublets in which Ala-Ala and Pro-Pro sequences get repeated 6 times.
- Fetuin-A also contains collagen-like sequences that are found in the complement component C1q.

SYNTHESIS:

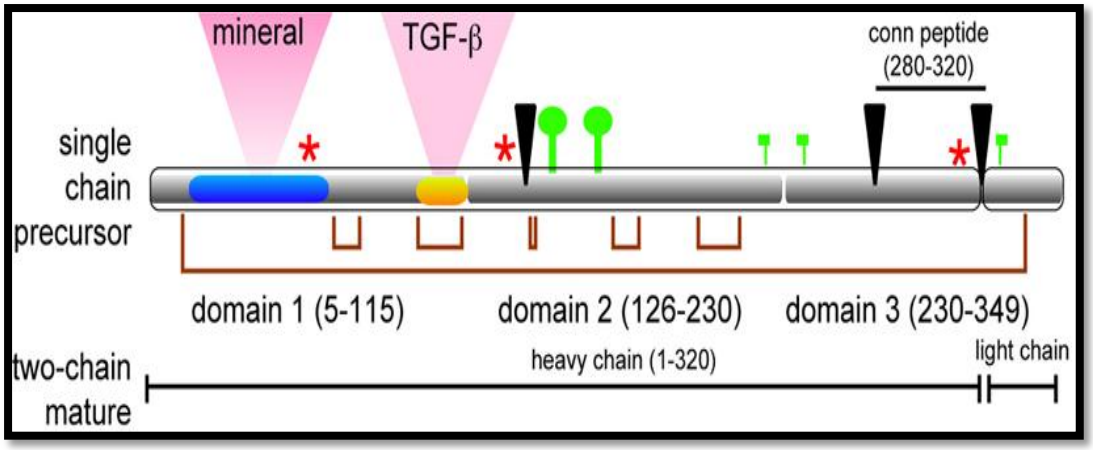
Fetuin-A is mainly synthesized by the hepatic parenchymal cells. The human gene was mapped to the region 3q21-29 of chromosome 3. The mode of inheritance is autosomal dominant[61].

- Fetuin-A is a typical secretory protein, biosynthesized from a single mRNA transcript in the form of a precursor protein of 367 aminoacid residues.
- Pre-Fetuin-A comprises a transient signal sequence of 18 residues, followed by the sequence of ‘A’ chain (positions 1-282 of matured protein), a connecting peptide (positions 283-322) and the ‘B’ chain (position 323-349).

- In a post translational step, the connecting peptide is cleaved by limited proteolysis which produces ‘A’ and ‘B’ chains that are joined together by a disulfide bond[62].
- Fetuin-A is also post translationally modified by the addition of five carbohydrate side chains – three of them are attached via O-glycosidical and two via N-glycosidical linkages. Fetuin-A is also phosphorylated at serine residues, particularly on serine-120 located in the ‘A’ chain and on serine-312 located in the connecting peptide[63].

Figure 8 shows the post-translational modification of Fetuin-A. Black wedges indicate proteolytic cleavage sites, red asterisks indicate serine phosphorylation sites, large green beacons indicate N-glycosylation sites and small green beacons indicate O-glycosylation sites. Binding regions for apatite and TGF-β like growth factors are shown as pink triangles.

Fig.8: POST-TRANSLATIONAL MODIFICATION OF FETUIN-A



BIOLOGICAL FUNCTIONS OF FETUIN-A:

A wide range of biological functions have been proposed for Fetuin-A.

(i) OSTEOGENESIS:[64,65]

- Fetuin-A is one of the most abundant non-collgenous protein found in the bone. The concentration of Fetuin-A present in one gram of bone is ~ 1 mg. It binds strongly to apatite which is the mineral phase of the bone and is concentrated selectively from the serum to the apatite. Further mineralization of type I collagen fibrils is promoted by Fetuin-A.
- Fetuin-A is concentrated up to 300 fold in the matrix of adult and fetal bone compared to other glycoproteins. Fetuin-A concentration progressively decreases in bone throughout childhood to adult life.
- Fetal bone was found to contain atleast 10 times more Fetuin-A than the adult bone, while neonatal bone contains 7 times more Fetuin-A than does the adult bone. The reasons for low concentrations of serum Fetuin-A in adulthood may be,

➢ Bone mineralization starts in utero and rapid bone growth and mineralization occur soon after birth. During this time, Fetuin-A may be needed critically as a mineral chaperone to overcome pathological mineralization and also to help in physiologic bone mineralization. This

results in selective concentration of Fetuin-A from serum to the apatite in bone, thereby reducing its serum concentration.

(ii) INHIBITION OF PATHOLOGICAL CALCIFICATION:[66,67]

- Fetuin-A is a systemic protein inhibitor of calcification and is present throughout the extra cellular space.
- ~ 50% of calcification inhibitory capacity of the human plasma is contributed by Fetuin-A.
- Formation of fetuin-mineral complex inhibits the precipitation of basic calcium phosphate (BCP), thereby preventing unwanted calcification.
- One Fetuin-A molecule can bind six calcium ions. BCP precipitation inhibition activity resides in the cystatin-like domain D_1 of Fetuin-A. This domain harbors an EF-hand-like Ca^{2+} binding motif.

MECHANISM OF INHIBITION:

- In the serum, Fetuin-A stabilizes calcium and phosphate and prevents their precipitation by binding BCP. In this process, it forms transiently soluble, colloidal complexes called calciprotein particles, which are 30-150nm in diameter. Fetuin-A coating of BCP nuclei will delay the growth of insoluble crystals which in turn favours mobilization and removal of insoluble calcium salts in the calciprotein particles by phagocytosis[68].

Figure 9: CALCIPROTEIN PARTICLE (CPP)

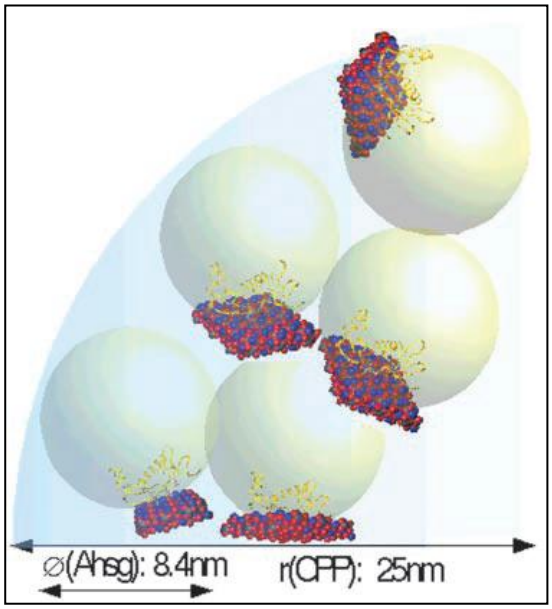


Figure 10: DOMAIN D1 OF FETUIN-A BINDING HYDROXYAPATITE

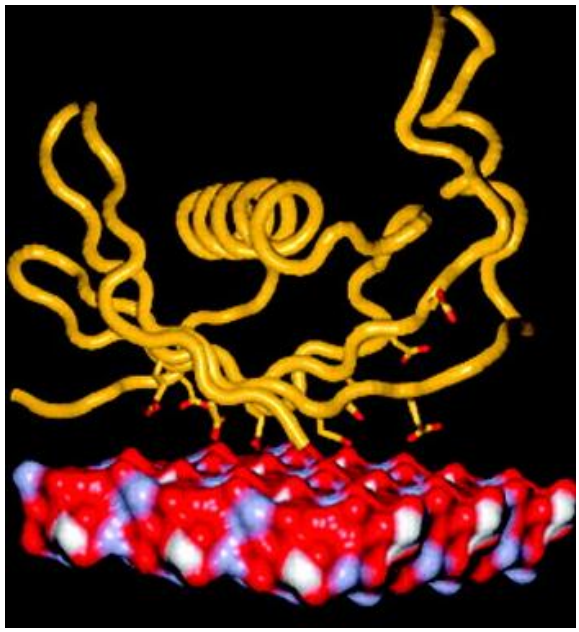


Figure 9 shows the hypothetical model of a calciprotein particle (large sphere quadrant) containing upto 100 globular Fetuin-A molecules (small spheres) with domain D1 bound to the apatite. Figure 10 shows Fetuin-A domain D1 (*top*) bound to the apatite crystals (*bottom*). Surface binding of calcium is mediated by the negative charges on the extended β sheet of the D1 domain resulting in high affinity binding despite the relatively low serum concentration of Fetuin-A.

Fetuin-A thus acts as a systemic inhibitor of pathological mineralization which complement the local inhibitors like matrix-Gla protein and pyrophosphate that act in a cell-restricted/ tissue-restricted fashion[69].

(iii) INHIBITION OF INFLAMMATION:[70,71]

Fetuin-A is an anti-inflammatory protein that can attenuate the inflammatory responses by the following mechanisms:

- Fetuin-A participates in macrophage deactivation. It enhances the uptake of pro-inflammatory cytokine synthesis inhibitors by macrophages, thereby preventing the morbid sequelae that would result from over production of pro-inflammatory cytokines.
- Spermine accumulates at the sites of inflammation and it inhibits the synthesis of cytokines by macrophages only in the presence of Fetuin-A.

- Fetuin-A also plays a role in mediating innate immunity.

➢ But the expression of Fetuin-A is negatively regulated by several pro-inflammatory cytokines which produces down regulation of its synthesis during inflammation; hence Fetuin-A is regarded as a "negative acute phase reactant".

(iv) OPSONIZATION:[73]

Fetuin-A also acts as an opsonin. It quenches the oxidative burst that occurs during the uptake of apatite crystals by neutrophils. It also facilitates marking and elimination of apoptotic neutrophils.

(v) INHIBITION OF INSULIN SIGNALLING:

Fetuin-A binds to the insulin receptor and inactivate the tyrosine kinase. Fetuin-A thereby inhibits the insulin-stimulated autophosphorylation of the insulin receptor and is associated with insulin resistance[72].

(vi) INHIBITION OF TGF-β SIGNALING:[74]

The aminoacid sequence of Fetuin-A is similar to that of TGF-β receptor. Hence Fetuin-A binds to TGF-β and prevents it from binding to its receptors thereby antagonizing TGF-β mediated anti-proliferative effects.

AIMS AND OBJECTIVES

AIM:

To estimate the levels of serum Fetuin-A in patients with CKD and to compare them with healthy controls.

OBJECTIVES:

1. To study the relationship of serum Fetuin-A with the inflammatory biomarkers, hsCRP and albumin
2. To evaluate the association of serum Fetuin-A with creatinine clearance
3. To find out the correlation of serum Fetuin-A with calcium-phosphorus levels and lipid profile

MATERIALS AND METHODS

The study was conducted at Thanjavur Medical College hospital, Thanjavur after getting approval from the ethical committee.

80 patients (55 males and 25 females) were selected as cases from the outpatients and wards of the department of Nephrology. 80 age and gender matched healthy individuals were selected as controls.

INCLUSION CRITERIA:

- Patients with established diagnosis of CKD
- Age > 18 years

EXCLUSION CRITERIA:

- Acute/Chronic inflammatory diseases
- Previous history of cerebrovascular diseases
- Patients who underwent renal transplant
- Acute kidney injury
- Nephrotic syndrome
- Patients on lipid lowering drugs and calcium/phosphate binders
- Malignancy
- Patients on immunotherapy/immunosuppressive treatment

Informed consent was obtained from all subjects prior to the study. Blood samples were collected from them after an overnight fasting of 12 hours. Under aseptic precautions, 5 ml of venous blood sample was collected

from each patient. After retraction of the clot, the samples were centrifuged at 2000 rpm for 15 minutes for separation of serum.

An aliquot of the serum was taken for the estimation of Fetuin-A and stored at -20°C in the deep freezer. The remaining serum was used for estimation of glucose, urea, creatinine, calcium, phosphorus, hsCRP, albumin, total cholesterol, triglycerides and HDL-C.

ANALYSIS OF BLOOD SAMPLES:

The serum collected above was used for the estimation of the following parameters.

A. ESTIMATED PARAMETERS:

1. Fetuin-A – Enzyme immunoassay
2. hsCRP – Turbidimetric immunoassay
3. Urea – Urease (GLDH) method
4. Creatinine – Modified Jaffe's method
5. Albumin - BromoCresol Green dye binding method
6. Calcium – Arsenazo method
7. Phosphorus – UV Molybdate method
8. Total cholesterol – Cholesterol-Oxidase – PAP method
9. Triglycerides – GPO – PAP method
10. HDL-C – Phosphotungstate/Magnesium precipitation method
11. Glucose – Glucose-Oxidase/Peroxidase method

B. CALCULATED PARAMETERS:

1. LDL-C and VLDL-C were calculated using Friedwald's formula:

   VLDL-C = TGL/5

   LDL-C = TC – [HDL-C + (TGL/5)]

2. Creatinine clearance (C_{cr}) was calculated using Cockcroft-Gault formula:

$$Ccr = \frac{[140 - AGE\,(Years)] * WEIGHT\,(Kg)}{72 * S.CREATININE\,(mg/dl)}$$

   For females,

$$Ccr = \frac{[140 - AGE\,(Years)] * WEIGHT\,(Kg)}{72 * S.CREATININE\,(mg/dl)} * 0.85$$

3. Calcium-phosphorus product (Ca X P) was calculated by multiplying the serum calcium and phosphorus values.

ESTIMATION OF SERUM FETUIN-A:

Enzyme linked immunosorbent assay (ELISA) kit obtained from R&D systems was used.

PRINCIPLE OF THE ASSAY:

Quantitative sandwich enzyme immunoassay technique is employed. A monoclonal antibody specific for Fetuin-A has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Fetuin-A present is bound by the immobilized antibody. An enzyme-linked monoclonal antibody specific for Fetuin-A is added to the wells after washing away any unbound substances. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of Fetuin-A bound. The colour development is stopped and the intensity of the colour is measured.

MATERIALS PROVIDED IN THE KIT:

1. Fetuin-A microplate: 96 well polystyrene microplate coated with a mouse monoclonal antibody against Fetuin-A
2. Fetuin-A standard: 1000 ng of recombinant human Fetuin-A in a buffered protein base with preservative; lyophilized
3. Fetuin-A conjugate: 21 ml of a monoclonal antibody against Fetuin-A conjugated to horseradish peroxidase with preservatives
4. Calibrator diluent RD5-26 concentrate: 21 ml of a concentrated buffered protein base with preservatives

5. Assay diluent RD1X: 11 ml of a buffered protein base with preservative
6. Wash buffer concentrate: 21 ml of a 25-fold concentrated solution of buffered surfactant with preservative
7. Colour Reagent A: 12.5 ml of stabilised hydrogen peroxide
8. Colour Reagent B: 12.5 ml of stabilized chromogen (tetramethyl benzidine)
9. Stop solution: 6 ml of 2 N sulphuric acid

SAMPLE PREPARATION:

Samples require a 4000-fold dilution. 10µL of the sample was added to 990 µL of the Calibrator Diluent RD5-26 (1X) and 25 µL of this diluted sample was added to 975 µL Calibrator Diluent RD5-26 (1X) to get a 4000-fold dilution.

REAGENT PREPARATION:

All the reagents were brought to room temperature before use.

Wash buffer: 20 ml of wash buffer concentrate was diluted into distilled water to prepare 500 ml of wash buffer.

Substrate solution: Colour reagents A and B were mixed together in equal volumes within 15 minutes of use and protected from light.

Calibrator Diluent RD5-26 (IX): 5 ml of Calibrator Diluent RD5-26 was diluted into 45 ml of distilled water to prepare 50 ml of Calibrator Diluent RD5-26 (IX).

Fetuin-A standard:

Fetuin-A standard was reconstituted with 2 ml of calibrator diluent RD5-26(IX), which produces a stock solution of 500 ng/ml. A dilution series was produced as follows:

STANDARD TUBES	CALIBRATOR DILUENT RD5-26(IX)	AMOUNT OF FETUIN-A STANDARD (µl)	CONCENTRATION OF FETUIN-A STANDARD(ng/ml)
1	250 µl	-	0
2	250 µl	250 µl of tube no. 3	7.8
3	250 µl	250 µl of tube no. 4	15.6
4	250 µl	250 µl of tube no. 5	31.3
5	250 µl	250 µl of tube no. 6	62.5
6	250 µl	250 µl of tube no. 7	125
7	250 µl	250 µl of stock	250
8	-	250 µl of stock	500

ASSAY PROCEDURE:

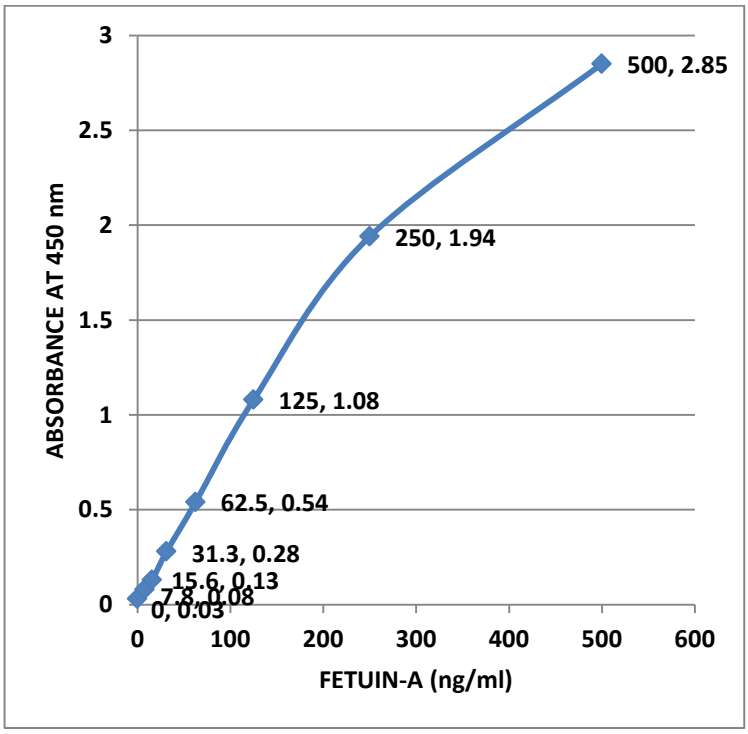
1. 100 µL Assay Diluent RD1X was added to each well
2. 50 µL of standard and sample was then added to each well and incubated for 2 hours at each temperature. Wells were aspirated and washed 4 times
3. 200 µL conjugate was added to each well and incubated for 2 hours at room temperature. Wells were aspirated and washed 4 times

4. 200 µL substrate solution was added to each well and incubated for 30 minutes at room temperature and protected from light
5. 50 µL of stop solution was added to each well and read at 450 nm within 30 minutes

CALIBRATION GRAPH:

By plotting the mean absorbance for each of the standards on the y-axis against the concentration of Fetuin-A on the x-axis, a calibration graph was constructed (Figure 11).

Figure 11: STANDARD GRAPH FOR FETUIN-A



SENSITIVITY:

The minimum detectable dose of Fetuin-A was 0.16-1.74 ng/ml.

REFERENCE RANGE: 0.5-1 g/L

ESTIMATION OF SERUM hs-CRP:

METHOD: Turbidimetric immunoassay (Biosystems)

PRINCIPLE:

Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with anti-human C-reactive protein. The latex agglutination is proportional to the concentration of CRP and can be measured by turbidimetry.

REAGENTS REQUIRED:

1. REAGENT A: Glycine buffer 0.1mol/L, sodium azide 0.95 g/L, pH 8.6
2. REAGENT B: Suspension of latex particles coated with anti-human CRP antibodies, sodium azide 0.95 g/L
3. hsCRP standard: 13.8 mg/L

REAGENT PREPARATION:

- Working reagent: Reagent B was poured into reagent A vial and mixed.
- **hsCRP standard:**

  hsCRP standard was reconstituted with 5 ml of distilled water which produces a stock solution of 13.8 mg/L. A dilution series was prepared as follows:

STANDARD TUBES	NORMAL SALINE	AMOUNT OF hsCRP STANDARD (µl)	CONCENTRATION OF hsCRP STANDARD (mg/L)
1	100 µl	-	0
2	100 µl	100µl of tube no. 3	0.43
3	100 µl	100 µl of tube no. 4	0.86
4	100 µl	100 µl of tube no. 5	1.725
5	100 µl	100 µl of tube no. 6	3.45
6	100 µl	100 µl of stock	6.9
7	-	100 µl of stock	13.8

PROCEDURE:

Reagents and samples were brought to room temperature prior to use.

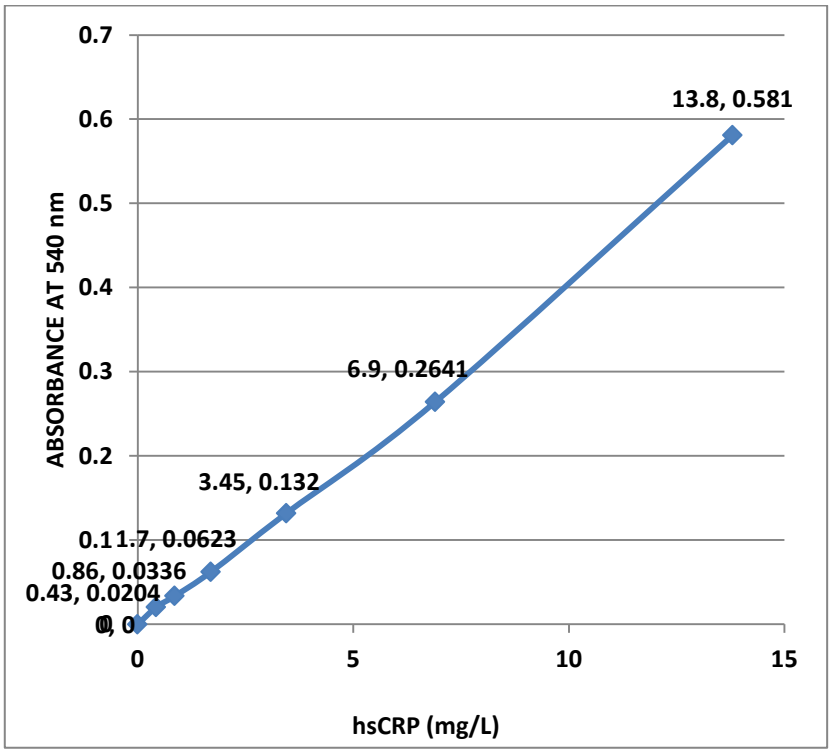
1) Zero correction of the instrument was done with distilled water.
2) Working reagent 1.5 ml and serum 20 µl were mixed in a cuvette.
3) Immediately the cuvette was inserted into the instrument and stop watch started.
4) The absorbance was recorded at 540 nm after 10 seconds (A_1) and after 5 minutes (A_2)

CALIBRATION:

The absorbance difference (A_2 - A_1) of each point of the calibration curve was calculated and the values found were plotted against the hsCRP

concentration and the calibration graph was constructed (Figure 12). The hsCRP concentration in the sample was calculated by interpolation of its absorbance (A_2 - A_1) on the calibration curve.

Figure 12: STANDARD GRAPH FOR hsCRP



DETECTION LIMIT: 0.06 mg/L

MEASUREMENT INTERVAL: 0.06-15 mg/L

REFERENCE VALUES:

VALUES (mg/L)	CARDIOVASCULAR RISK
<1	Low
1-3	Moderate
>3	High

ESTIMATION OF UREA:

METHOD: Urease-GLDH method

PRINCIPLE:

Urea in the sample is hydrolysed to ammonia and carbon di oxide by urease. Glutamate dehydrogenase (GLDH) catalyse the second reaction that converts ammonia and α-ketoglutarate to glutamate and water with the concurrent oxidation of reduced NADH to NAD. Two moles of NADH are oxidized for each mole of urea present.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2-Oxoglutarate} \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+$$

The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample.

REAGENT COMPOSITION:

Reagent 1: α-ketoglutaric acid 99.8 mmol/L, Urease 23.5 KU/L, GLDH 3.5 KU/L, Adenosine diphosphate 7.6 mmol/L, Sodium azide 0.2%

Reagent 2: NADH 2.95 mmol/L, Sodium azide 0.1%

REAGENT PREPARATION:

Working reagent was prepared by mixing 4 parts of reagent 1 with one part of reagent 2.

PROCEDURE:

PIPETTE INTO TUBES	BLANK	STANDARD	TEST
Working Reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Test	-	-	10 µl

The tubes were mixed well and the absorbance was read after 30 seconds (A_1) and 60 sec (A_2) at 340 nm.

CALCULATION:

$\Delta A = A_2 - A_1$

$$\text{Urea (mg/dl)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \text{ x Concentration of standard (50 mg/dl)}$$

LINEARITY: The method is linear upto 200 mg/dl

REFERENCE INTERVAL: 15-30 mg/dl

ESTIMATION OF SERUM CREATININE:

METHOD:

Modified Jaffe's method

PRINCIPLE:

Creatinine reacts with alkaline picrate to produce an orange-yellow colour. The intensity of the orange-yellow colour formed is directly proportional to concentration of creatinine and is measured photometrically at 500-520 nm.

REAGENT COMPOSITION:

REAGENT NO.	COMPOSITION	CONCENTRATION
1	Picric acid	25.8 mmol/L
2	Sodium hydroxide	95 mmol/L

CREATININE STANDARD: 2 mg/dl

REAGENT PREPARATION: Equal volumes of reagent 1 and reagent 2 were mixed and waited for 15 minutes before use.

PROCEDURE:

PIPETTE	STANDARD	TEST
Working Reagent	1000 µl	1000 µl
Standard	100 µl	-
Test	-	100 µl

The tubes were mixed well and the initial absorbance A_1 [20 seconds after mixing] and final absorbance A_2 [80 seconds after mixing] were read at 505 nm.

CALCULATION:

$\Delta A = A_2 - A_1$

$$\text{Serum Creatinine(mg/dl)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \text{ x Concentration of standard (mg/dl)}$$

LINEARITY: The assay is linear upto 20 mg/dl

REFERENCE RANGE:

Males: 0.7-1.4 mg/dl

Females: 0.6-1.2 mg/dl

ESTIMATION OF SERUM ALBUMIN:

METHOD:

BromoCresol Green dye binding method

PRINCIPLE:

Albumin at pH 4.2, binds with BromoCresol Green (BCG) which causes a shift in absorbance of the yellow BCG dye. The blue-green colour formed is measured photometrically between 580-630 nm with maximum absorbance at 625 nm and is proportional to the albumin concentration.

REAGENT COMPOSITION:

BromoCresol Green	0.08 mmol/L
Succinate buffer (pH 4.2 ± 0.1 at 25° C)	50 mmol/L
Sodium azide	1 gm/L

Albumin Standard: 3.6 g/dl

PROCEDURE:

PIPETTE INTO TUBES MARKED	BLANK	STANDARD	TEST
Albumin reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Test	-	-	10 µl

The tubes were mixed well and the absorbance of standard and test were read at 630 nm (580-630 nm) against reagent blank after 1 minute incubation at 37° C.

CALCULATION:

$$\text{Serum Albumin (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Concentration of standard (g/dl)}$$

LINEARITY: The assay is linear upto 6 g/dl.

NORMAL VALUE: 3.5-5 g/dl

ESTIMATION OF SERUM CALCIUM:

METHOD: Arsenazo method

PRINCIPLE:

Arsenazo III combines with calcium ions at pH 6.5 to form a coloured chromophore, the absorbance of which is measured at 650 nm and is proportional to calcium concentration.

REAGENT COMPOSITION:

ACTIVE INGREDIENTS	CONCENTRATION
Arsenazo III	0.20 mmol/l
Imidazole buffer (pH 6.5±0.1)	100 mmol/l

Calcium standard: 10 mg/dl

PROCEDURE:

PIPETTE INTO TUBES MARKED	BLANK	STANDARD	TEST
Calcium reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Test	-	-	10 µl

The tubes were mixed well, incubated at 37° C for 5 minutes and the absorbances were measured at 540 nm.

CALCULATION:

$$\text{Serum Calcium (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x } 10$$

LINEARITY: The assay is linear upto 16 mg/dl

REFERENCE RANGE: 9-11 mg/dl

ESTIMATION OF SERUM PHOSPHORUS:

METHOD: UV Molybdate method

PRINCIPLE:

Inorganic phosphorus reacts with ammonium molybdate in acidic medium to form phosphomolybdate complex. This colourless complex is measured at 340 nm and is directly proportional to the inorganic phosphorus concentration in the sample.

REAGENT COMPOSITION:

REAGENT NO.	REAGENT	COMPOSITION	CONCENTRATION
1	Molybdate reagent	Ammonium Molybdate Surfactant	0.3 mM/L
2	Sample blank reagent	Sodium chloride Preservative	154 mM/L
3	Phosphorus Standard	Potassium dihydrogen phosphate	5 mg/dl

PROCEDURE:

PIPETTE INTO TUBES MARKED	REAGENT BLANK	STANDARD	TEST
Serum	-	-	10 µl
Reagent 3	-	10 µl	-
Reagent 1	1000 µl	1000 µl	1000 µl

The tubes were mixed well and incubated at 37° C for 5 minutes. After blanking the analyser with reagent blank, the absorbances were measured at 340 nm.

CALCULATION:

$$\text{Serum Phosphorus(mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x } 5$$

REFERENCE RANGE:

2.5-4.5 mg/dl

TOTAL CHOLESTEROL ESTIMATION

METHOD: Cholesterol oxidase - PAP, endpoint

PRINCIPLE:

1. Cholesterol ester +Water $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acid

2. Cholesterol + Oxygen $\xrightarrow{\text{Cholesterol oxidase}}$ Cholest-4-en-3one + H_2O_2

3. $2H_2O_2$ + 4AAP+ Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + $4H_2O$

- 4AAP- 4 amino antipyrine
- Absorbance of quinoneimine formed is directly proportional to cholesterol concentration.
- Cholesterol standard – 200mg/dl
- Sample: Unhemolysed serum

REAGENT COMPOSITION:

Goods buffer (pH-6.4) : 100mmol/L

Cholesterol oxidase : >100U/L

Cholesterol esterase : >200U/L

Peroxidase : >3000U/L

4-Aminoantipyrine : 0.3mmol/L

Phenol : 5mmol/L

PROCEDURE:

REAGENTS	BLANK	STANDARD	TEST
Working reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

The tubes were mixed well and incubated for 10 min at room temperature. The absorbance of the test and standard were read against reagent blank at wavelength of 505 nm.

CALCULATION:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Concentration of standard (mg/dl)}$$

REFERENCE RANGE:

SERUM/PLASMA	mg/dl
2-12 months	60-190
≥ 1 year	110-230
Adults	< 200

Linearity-upto 700mg/dl

ESTIMATION OF TRIGLYCERIDES:

METHOD:

Colorimetric, enzymatic method with glycerol phosphate oxidase (GPO-PAP method).This reagent is based on the method of Wako and the modifications by McGowan et al. and Fossati et al.

PRINCIPLE:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{Glycerol kinase}} \text{Glycerol 3 phosphate} + \text{ADP}$$

$$\text{Glycerol 3 phosphate} + O_2 \xrightarrow{\text{Glycerol Phosphate Oxidase}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4AAP} + \text{3, 5-DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 2H_2O$$

ATP - Adenosine Tri Phosphate, 4AAP - 4Amino Anti Pyrine, DHBS - 3, 5 Dichloro-2Hydroxy Benzene Sulfonate

The intensity of Quinoneimine dye formed is proportional to the triglyceride concentration in the sample when measured at 505 nm (500-540nm).

Triglycerides standard concentration- 200mg/dl

REAGENT COMPOSITION:

REAGENT 1(Enzymes / Chromogen):

Lipoprotein lipase	4000 U/L
4-Amino antipyrine	0.4 mmol/L
ATP	2mmol/L
Glycerol kinase	1500 U/L
Peroxidase	2200 U/L
Glycerol Phosphate Oxidase	4000 U/L

REAGENT 2:

Pipes buffer, pH-7.0: 40mmol/L

DHBS: 0.2mmol/L

Magnesium salt: 2.5mmol/L

WORKING REAGENT PREPARATION:

The working reagent was prepared by mixing 4 parts of R1 with 1 part of R2, stable for 90 days at 2-8°C.

PROCEDURE:

REAGENTS	BLANK	STANDARD	TEST
Working reagent	1000µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mixed and incubated for 10min. Absorbance read at 505nm for standard and sample against reagent blank.

CALCULATION:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Concentration of standard (mg/dl)}$$

REFERENCE VALUES: 25-160 mg/dl

LINEARITY: upto 1000mg/dl

HDL CHOLESTEROL ESTIMATION:

METHOD: Phosphotungstic acid/Magnesium precipitation method, endpoint

PRINCIPLE:

Chylomicrons, VLDL-C and LDL-C are precipitated from the serum by phospho tungstate in the presence of divalent cations such as Magnesium. The HDL cholesterol reminds unaffected in the supernatant and is estimated using cholesterol reagent.

$$\text{Serum/plasma} \xrightarrow[Mg^{2+}]{\text{Phosphotungstic acid}} \underset{\text{(supernatant)}}{\text{HDL-C}} + \underset{\text{(Precipitate)}}{\text{(LDL-C+VLDL-C+Chylomicrons)}}$$

REAGENT COMPOSITION:

Reagent1: Precipitating reagent

Phosphotungstic acid	2.4mmol/l
Magnesium chloride	40mmol/l

HDL cholesterol standard – 25mg/dl

PRECIPITATION:

Precipitation of LDL-C, VLDL-C and Chylomicrons were done as follows:

Pipette	Volume
Test	250µl
Precipitating reagent	500 µl

The tubes were mixed well and the reaction mixture was allowed to stand for 10 min at room temperature, centrifuged at 4000 rpm for 10 min and a clear supernatant was obtained. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

PROCEDURE:

REAGENTS	BLANK	STANDARD	TEST
Cholesterol working reagent	1000µl	1000 µl	1000µl
Distilled water	50 µl	-	-
HDL standard	-	50 µl	-
Supernatant	-	-	50 µl

Mixed well and incubated for 10 min at room temperature. The absorbance of the standard and the test samples were read at 505 nm against reagent blank.

Calculation:

$$\text{HDL cholesterol(mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Concentration of standard} \times \text{dilution factor}$$

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x 25 x 3}$$

LINEARITY**: -** upto 125mg/dl

NORMAL VALUES:

Males - 30 to 65mg/dl

Females – 35 to 80mg/dl

ESTIMATION OF GLUCOSE

METHOD: Trinder's method, end point/fixed time

PRINCIPLE:

Glucose in the sample is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyses oxidative coupling of 4 –amino antipyrine (4-AAP)with phenol to yield a colored quinoneimine complex, with absorbance proportional to concentration of glucose in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol+4 AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 2\ H_2O_2$$

Glucose standard: 100mg/dl

Specimen: Fresh unhemolysed serum used

ASSAY PROCEDURE:

REAGENTS	BLANK	STANDARD	TEST
Sample	-	-	10µl
Standard	-	10µl	-
Distilled water	10µl	-	-
Enzyme reagent	1ml	1ml	1ml

The tubes were mixed well after each addition and incubated at 37°C for 5 min. The absorbance was read at 505nm/670nm.

CALCULATION:

$$\text{GLUCOSE (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Concentration of standard (mg/dl)}$$

Linearity: upto 500mg/dl by endpoint method.

NORMAL VALUES: Glucose (Fasting): 65-110 mg/dl

Glucose (Postprandial): 90-140mg/dl

RESULTS AND STATISTICAL ANALYSIS:

MASTER CHART I - CONTROLS

S.No	AGE years	SEX	HT m	WT Kg	BMI Kg^2/m^2	SBP mmHg	DBP mmHg	FBG mg/dl	UREA mg/dl	CREAT mg/dl	Ccr ml/min	hsCRP mg/L	FETUIN-A g/L	CAL mg/dl	PHOS mg/dl	CaXP mg^2/dl^2	ALB g/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl
1	38	M	1.68	62	21.97	110	70	86	20	0.9	97.59	0.83	0.58	9.6	3.04	29.18	3.8	168	110	42	22	104
2	31	M	1.64	68	25.28	104	80	90	22	1.1	93.59	0.29	0.618	10.2	2.67	27.23	3.67	186	150	46	30	110
3	37	M	1.6	72	28.13	120	76	80	18	0.9	114.4	0.21	0.734	9.4	4.1	38.54	4.25	164	122	46	24.4	93.6
4	35	M	1.56	66	27.12	90	70	98	28	0.9	106.9	0.58	0.853	10.6	2.93	31.06	4.05	150	112	45	22.4	82.6
5	37	M	1.7	70	23.53	110	80	100	20	1.1	91.04	0.81	0.612	10.9	3.52	38.37	3.64	180	135	42	27	111
6	26	M	1.68	68	24.09	114	76	102	24	0.8	134.6	0.46	0.528	9.87	3.15	31.09	4	174	140	40	28	106
7	32	M	1.7	65	22.49	100	70	78	24	0.8	121.9	0.89	0.59	10.4	3.6	37.26	4.5	150	124	38	24.8	87.2
8	33	M	1.56	67	27.53	96	80	82	18	0.9	110.6	0.33	0.606	10.9	3.28	35.75	3.74	160	150	46	30	84
9	35	M	1.56	55	22.6	112	80	96	26	0.8	100.3	0.42	1.13	10.5	2.97	31.19	4.65	176	142	42	28.4	106
10	34	M	1.62	68	25.91	98	74	72	20	1	100.1	0.22	0.71	9.6	3.8	36.48	3.58	178	135	50	27	101
11	36	M	1.65	60	20.57	120	80	78	20	0.9	96.3	0.56	0.52	10.3	2.83	29.15	3.96	184	140	40	28	116
12	38	M	1.68	67	23.74	116	76	100	22	1	94.92	0.92	0.56	9.2	4.3	39.56	4.04	160	138	45	27.6	87.4
13	36	M	1.56	65	26.71	90	70	104	28	1	93.89	0.81	0.819	10.6	2.82	29.89	4	208	122	41	24.4	143
14	28	M	1.65	56	20.57	118	80	84	20	0.9	96.79	0.67	0.72	9.37	3.26	30.55	3.68	188	120	40	24	124
15	58	M	1.69	68	23.81	110	78	90	26	0.8	96.81	0.72	0.618	10.5	3.7	38.85	3.9	175	154	48	30.8	96.2
16	68	M	1.68	73	25.86	100	70	86	26	0.8	91.25	0.32	0.63	10.3	3.15	32.45	4.2	182	160	44	32	106
17	64	M	1.58	69	27.64	96	76	92	24	0.8	91.04	0.88	0.59	9.5	4.2	39.9	4.24	168	110	42	22	104
18	61	M	1.63	60	19.2	126	74	76	24	0.7	94.05	0.41	0.614	9.23	3.5	32.31	4.13	200	124	45	24.8	130
19	62	M	1.67	68	24.38	90	70	84	28	0.8	92.08	0.29	0.597	10.5	3.27	34.34	3.7	165	115	42	23	100
20	53	M	1.7	68	23.53	108	72	98	22	0.9	91.3	0.47	0.508	10.7	3.25	34.78	3.6	152	148	40	29.6	82.4
21	63	M	1.52	68	29.43	110	80	86	26	0.8	90.9	0.73	0.57	9.8	3.63	35.57	3.82	188	134	46	26.8	115
22	61	M	1.57	67	27.18	100	74	100	24	0.8	91.89	0.91	0.542	9.87	2.85	28.13	3.67	164	140	38	28	98
23	62	M	1.56	70	28.76	96	70	78	24	0.8	94.79	0.53	0.519	10.2	3.27	33.35	4.65	166	128	45	25.6	95.4
24	61	M	1.63	72	27.1	94	72	104	20	0.9	87.78	0.25	0.639	10.8	3.09	33.37	3.8	194	136	40	27.2	127
25	62	M	1.69	68	23.81	106	80	92	18	0.8	92.08	0.36	0.753	9.6	3.3	31.68	4.01	202	120	36	24	142
26	54	M	1.55	65	27.06	114	86	88	22	0.8	97.05	0.29	0.724	9.79	3.22	31.52	4.89	154	130	42	26	86
27	45	M	1.7	62	21.45	130	80	76	28	0.9	90.9	0.66	0.661	9.9	3.16	31.28	3.98	168	142	38	28.4	102
28	50	M	1.6	66	25.78	124	78	100	28	0.8	103.1	0.89	0.87	10.1	3.33	33.63	3.64	165	138	40	27.6	97.4

S.No	AGE years	SEX	HT m	WT Kg	BMI kg^2/m^2	SBP mmHg	DBP mmHg	FBG mg/dl	UREA mg/dl	CREAT mg/dl	Ccr ml/min	hs-CRP mg/L	FETUIN-A g/L	CAL mg/dl	PHOS mg/dl	CaXP mg^2/dl^2	ALB g/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl
29	43	M	1.56	62	25.48	116	70	98	24	0.9	92.81	0.73	0.572	9.85	2.97	29.25	3.7	170	155	43	31	96
30	55	M	1.65	56	20.57	110	76	94	20	0.8	82.64	0.45	0.648	9.34	3.14	29.33	3.85	182	146	44	29.2	109
31	52	M	1.58	60	21.63	120	76	84	20	0.8	91.67	0.76	0.86	9.5	3.98	37.81	4.13	152	142	42	28.4	81.6
32	45	M	1.55	58	19.98	108	72	84	24	0.8	95.66	0.38	0.614	10.4	3.61	37.54	4.24	162	130	40	26	96
33	52	M	1.67	56	20.08	90	70	76	22	0.7	97.78	0.22	0.638	9.6	4.02	38.59	4.6	172	158	48	31.6	92.4
34	42	M	1.52	54	23.37	112	84	98	28	0.8	91.88	0.46	0.64	9.87	3.17	31.29	3.8	192	128	38	25.6	128
35	40	M	1.56	66	27.12	88	70	74	26	0.8	114.6	0.34	0.632	9.54	3.53	33.68	3.56	180	108	38	21.6	120
36	47	M	1.63	63	23.71	100	74	88	18	0.9	90.42	0.98	0.558	10.5	3.67	38.54	3.84	172	100	42	20	110
37	41	M	1.68	60	21.26	120	78	88	20	0.8	103.1	0.49	0.62	9.51	3.07	29.2	3.71	200	150	45	30	125
38	54	M	1.56	68	27.94	104	80	100	20	0.9	90.25	0.65	0.653	9.16	3.22	29.5	3.78	170	122	40	24.4	106
39	51	M	1.64	62	23.05	110	74	104	22	0.8	95.8	0.97	0.792	9.97	3.29	32.8	4.03	148	134	42	26.8	79.2
40	50	M	1.52	64	27.7	114	80	102	28	0.8	100	0.73	0.91	9.88	3.6	35.57	3.63	196	126	38	25.2	133
41	52	M	1.67	76	27.25	90	70	86	26	1	92.89	0.26	0.823	10.3	3.37	34.71	3.68	182	114	40	22.8	119
42	51	M	1.56	66	27.12	98	72	78	20	0.9	90.65	0.87	0.63	9.9	3.18	31.48	3.98	188	140	42	28	118
43	52	M	1.62	60	22.86	120	76	78	22	0.8	91.67	0.26	0.96	9.12	3.4	31.01	4.25	176	132	46	26.4	104
44	43	M	1.6	71	27.73	110	70	86	22	1	95.65	0.92	1.07	10.3	3.07	31.56	4.34	160	106	43	21.2	95.8
45	47	M	1.72	68	22.99	104	80	98	28	0.9	97.59	0.35	0.88	9.3	3.9	36.27	3.94	165	102	40	20.4	105
46	42	M	1.53	63	26.91	108	78	100	24	0.8	107.2	0.63	0.935	9.78	3.64	35.6	3.68	178	112	46	22.4	110
47	40	M	1.66	65	23.59	120	74	84	24	0.9	100.3	0.38	0.81	10.5	3.36	35.28	4.42	160	100	38	20	102
48	62	M	1.54	67	28.25	110	70	84	20	0.8	90.73	0.82	0.56	9.6	3.7	35.52	4.26	182	128	40	25.6	116
49	62	M	1.67	68	24.38	126	80	100	28	0.8	92.08	0.57	1.08	9.2	4.13	38	4.7	170	124	36	24.8	109
50	45	M	1.72	55	18.59	116	74	100	18	0.8	90.71	0.47	0.834	10.3	3.06	31.58	3.6	168	165	48	33	87
51	48	M	1.6	64	25	100	72	88	22	0.9	90.86	0.95	0.928	9.67	3.51	33.94	3.78	156	120	42	24	90
52	61	M	1.57	66	26.78	90	70	82	22	0.7	103.5	0.72	0.95	10.3	3.42	35.36	4.36	164	110	40	22	102
53	62	M	1.64	67	24.91	120	78	78	18	0.8	90.73	0.24	0.61	9.51	3.16	30.05	4.09	158	132	43	26.4	88.6
54	46	M	1.62	65	24.77	106	74	92	28	0.8	106.1	0.28	0.713	10.3	3.27	33.78	3.76	196	126	45	25.2	126
55	42	M	1.58	56	22.43	96	70	98	26	0.8	95.28	0.41	0.78	9.8	3.76	36.85	3.65	172	146	35	29.2	108
56	30	F	1.67	60	21.51	120	76	74	26	0.9	101.9	0.76	0.969	9.78	3.18	31.1	4.5	166	158	40	31.6	94.4
57	33	F	1.52	50	21.64	100	70	100	20	0.8	92.88	0.39	0.94	10.5	3.39	35.6	4.32	182	134	42	26.8	113

S.No	AGE years	SEX	HT m	WT Kg	BMI kg^2/m^2	SBP mmHg	DBP mmHg	FBG mg/dl	UREA mg/dl	CREAT mg/dl	Ccr ml/min	hs-CRP mg/L	FETUIN-A g/L	CAL mg/dl	PHOS mg/dl	CaXP mg^2/dl^2	ALB g/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl
58	45	F	1.54	55	23.19	110	74	104	24	0.8	90.71	0.23	0.576	10.2	3.8	38.68	3.64	150	150	36	30	84
59	35	F	1.52	50	21.64	124	80	74	22	0.8	91.15	0.67	0.971	10.2	3.34	34.07	4.53	168	104	38	20.8	109
60	33	F	1.56	58	23.83	100	76	88	22	0.9	95.77	0.54	1.12	9.31	3.9	36.31	3.9	194	140	42	28	124
61	41	F	1.62	63	24.01	100	72	94	20	0.8	108.3	0.59	0.83	10	2.9	29	3.59	202	148	37	29.6	135
62	43	F	1.6	66	25.78	90	70	86	18	0.9	98.8	0.93	0.676	9.39	3.73	35.02	3.91	168	120	38	24	106
63	43	F	1.64	65	24.17	106	80	100	22	0.9	97.3	0.81	0.969	10.2	3.17	32.4	4.7	194	162	48	32.4	114
64	44	F	1.56	64	26.3	110	82	98	28	0.8	106.7	0.63	0.774	9.73	3.33	32.4	3.87	180	140	45	28	107
65	44	F	1.52	62	26.84	120	78	78	26	0.9	91.85	0.78	0.859	9.34	3.85	35.96	4.25	156	134	40	26.8	89.2
66	41	F	1.54	56	23.61	108	74	86	20	0.8	96.25	0.25	1.04	10.8	3.43	37.04	4.37	178	154	46	30.8	101
67	51	F	1.66	64	23.23	116	78	88	24	0.8	98.89	0.59	0.652	9.2	4.01	36.89	4.52	160	142	42	28.4	89.6
68	63	F	1.58	68	27.24	114	72	102	28	0.8	90.9	0.28	0.633	9.51	2.72	25.87	3.73	180	150	40	30	110
69	55	F	1.56	62	25.48	100	70	84	20	0.7	104.6	0.37	0.912	9.7	3.89	37.73	3.8	166	122	45	24.4	96.6
70	62	F	1.58	67	26.84	100	76	74	22	0.8	90.73	0.66	0.864	10.3	3.43	35.43	3.76	152	135	42	27	83
71	6	F	1.55	47	19.56	104	70	104	18	0.9	97.19	0.71	0.688	10.9	3.19	34.61	4.2	168	140	36	28	104
72	62	F	1.5	70	31.11	110	74	98	26	0.8	94.79	0.63	0.95	9.28	3.67	34.06	4.52	180	164	44	32.8	103
73	61	F	1.52	68	29.43	90	70	90	24	0.8	93.26	0.83	0.66	9.3	2.83	26.32	3.9	186	142	40	28.4	118
74	50	F	1.6	52	20.31	100	78	80	20	0.7	92.86	0.79	0.636	9.67	3.47	33.55	3.71	172	155	38	31	103
75	52	F	1.57	60	24.34	96	70	72	20	0.8	91.67	0.52	1.23	10.3	3.14	32.28	3.68	150	142	38	28.4	83.6
76	52	F	1.58	62	24.84	98	72	78	28	0.7	108.3	0.83	0.618	10	3.38	33.8	4.2	184	138	46	27.6	110
77	51	F	1.55	59	24.56	102	78	90	24	0.8	91.16	0.29	0.671	9.83	3.65	35.88	3.75	180	168	40	33.6	106
78	51	F	1.58	60	24.03	90	70	100	22	0.8	92.71	0.64	0.98	9.3	3.4	31.62	3.86	166	140	42	28	96
79	45	F	1.53	64	27.34	120	80	78	22	0.9	93.83	0.31	1.17	9.2	4	36.8	3.93	170	134	38	26.8	105
80	46	F	1.6	60	23.44	100	74	82	18	0.8	97.92	0.42	0.714	9.31	3.65	33.98	4.15	194	126	42	25.2	127

MASTER CHART II- CASES

S.No	AGE	SEX	HT m	WT Kg	BMI kg^2/m^2	SBP mmHg	DBP mmHg	FBG mg/dl	UREA mg/dl	CREAT mg/dl	Ccr ml/min	hsCRP mg/L	FETUIN-A g/L	CAL mg/dl	PHOS mg/dl	Ca X P mg^2/dl^2	ALB g/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl
1	55	M	1.7	55	19.03	110	80	130	110	2.03	31.99	3.96	0.55	9.75	4.2	40.95	3.34	196	160	38	32	126
2	45	M	1.62	45	17.15	130	70	116	90	1.94	30.61	2.99	0.455	9.23	4.38	40.43	3.2	160	230	35	46	79
3	53	M	1.68	48	17.01	128	84	86	104	1.7	34.12	3.28	0.49	10.5	4	42	3.59	200	170	33	34	133
4	42	M	1.62	43	16.38	170	100	148	88	1.9	30.8	0.91	0.463	9.51	3.9	37.09	3.67	160	200	40	40	80
5	39	M	1.58	55	22.03	100	80	134	122	2.32	33.26	4.1	0.47	10.32	3.7	38.18	3.5	184	188	36	37.6	110
6	63	M	1.64	60	22.31	144	96	140	118	2.13	30.13	3.52	0.442	10.69	4.23	45.22	3.03	176	180	34	36	106
7	38	M	1.72	67	22.65	120	60	96	90	1.8	52.73	2.04	0.493	10.3	3.94	40.58	2.9	160	168	36	33.6	90.4
8	42	M	1.56	58	23.83	152	94	80	98	2.1	37.59	2.63	0.52	9.26	4	37.04	4	182	240	43	48	91
9	58	M	1.68	72	25.51	120	80	102	136	5.1	16.08	7.3	0.31	9	4.5	40.5	2.99	168	230	32	46	90
10	57	M	1.57	55	22.31	126	82	110	124	3.2	19.81	5.55	0.44	8.9	4.9	43.61	3.72	160	170	40	34	86
11	42	M	1.6	50	19.53	140	90	128	140	4	17.01	2.51	0.41	8.87	4.72	41.87	3.06	176	210	35	42	99
12	61	M	1.67	50	17.93	160	80	76	134	3.24	16.93	4.7	0.402	9.8	4.74	46.45	3.39	150	192	38	38.4	73.6
13	58	M	1.58	52	20.83	180	110	118	130	2.89	20.49	6.91	0.38	9.5	5.57	52.92	3.4	168	184	34	36.8	97.2
14	50	M	1.66	45	16.33	116	86	72	128	3.22	17.47	3.28	0.393	9.67	4.93	47.67	3.25	174	208	36	41.6	96.4
15	38	M	1.54	40	16.87	100	70	86	94	2.3	24.64	2.91	0.42	8.73	5.1	44.52	3.13	164	187	32	37.4	94.6
16	35	M	1.7	86	29.76	124	82	120	126	5.7	22	8.89	0.338	9.3	5.33	49.57	2.57	160	176	42	35.2	82.8
17	38	M	1.62	53	20.2	140	70	114	130	4.9	15.32	5.58	0.353	9.18	5.42	49.76	2.7	172	152	40	30.4	102
18	65	M	1.67	72	25.82	110	70	72	114	3	25	2.9	0.412	10.1	4.62	46.66	2.98	176	166	36	33.2	107
19	73	M	1.73	52	17.37	120	80	106	90	2.81	17.22	10.4	0.37	10.9	4.79	52.21	3.18	184	208	32	41.6	110
20	75	M	1.78	60	18.94	150	90	132	96	2.3	23.55	7.61	0.313	9.79	4.82	47.19	3.03	172	180	46	36	90
21	32	M	1.62	55	20.96	150	100	110	104	3.14	26.27	8.7	0.329	8.6	5.2	44.72	3.42	182	192	42	38.4	102
22	55	M	1.67	65	23.31	114	76	146	92	2.7	28.42	4.53	0.32	8.72	5.59	48.74	3.1	162	152	34	30.4	97.6
23	47	M	1.73	57	19.05	118	80	86	88	2.5	29.45	3.69	0.241	10.5	4.83	50.72	2.91	174	184	38	36.8	99.2
24	65	M	1.64	43	15.99	152	84	112	108	3.8	11.79	7.44	0.15	8.9	5.9	52.51	2.59	150	200	38	40	72
25	65	M	1.53	35	14.95	126	82	120	92	3	12.15	10	0.282	9.33	5.55	51.78	3.1	180	180	28	36	116
26	64	M	1.58	56	22.43	134	80	104	136	4.6	12.85	9.18	0.31	8.87	6	53.22	2.98	160	208	34	41.6	84.4
27	75	M	1.48	35	15.98	120	90	72	116	2.79	11.33	10.32	0.303	8.57	6.33	54.25	3.38	168	184	46	36.8	85.2
28	74	M	1.54	45	18.97	128	82	110	104	3.1	13.31	8.73	0.18	8.53	7	59.71	3.06	190	181	26	36.2	128
29	35	M	1.65	52	19.1	90	70	82	160	9.1	8.333	7.31	0.252	9.13	5.69	51.95	2.9	164	176	38	35.2	90.8

S.No	AGE	SEX	HT m	WT Kg	BMI Kg^2/m^2	SBP mmHg	DBP mmHg	FBG mg/dl	UREA mg/dl	CREAT mg/dl	Ccr ml/min	hsCRP mg/L	FETUIN-A g/L	CAL mg/dl	PHOS mg/dl	Ca X P mg^2/dl^2	ALB g/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl
30	37	M	1.45	36	17.12	120	80	122	142	8.9	5.787	10.56	0.29	9.21	5.85	53.88	2.63	216	180	32	36	148
31	53	M	1.62	70	26.67	118	74	90	136	8.3	10.19	9.7	0.233	9.15	6.5	59.48	3.33	160	170	36	34	90
32	57	M	1.55	58	24.14	124	80	106	128	4.9	13.65	6.91	0.192	8.37	6.93	58	2.52	220	208	28	41.6	150
33	45	M	1.6	48	18.75	128	86	142	110	4.3	14.73	6.73	0.209	8.96	5.46	48.92	2.13	170	216	40	43.2	86.8
34	56	M	1.57	62	25.15	170	100	92	134	7.8	9.274	5.82	0.25	9.07	5.88	53.33	2.8	166	192	32	38.4	95.6
35	57	M	1.55	45	18.73	100	70	80	150	8.9	5.829	10.7	0.357	9.16	5.75	52.67	2.24	206	216	37	43.2	126
36	48	M	1.72	56	18.93	140	90	86	130	6.4	11.18	8.3	0.172	8.88	6.17	54.79	2.97	176	180	35	36	105
37	48	M	1.56	45	18.49	124	78	124	156	9.6	5.99	4.86	0.39	8.55	6.43	54.98	3.5	186	144	43	28.8	114
38	45	M	1.63	57	21.45	110	80	112	80	1.6	47.01	3.2	0.429	9.67	4.2	40.61	3.26	160	184	40	36.8	83.2
39	45	M	1.55	62	25.81	110	74	74	88	2.2	37.18	0.69	0.44	10.7	3.89	41.62	3.15	190	160	33	32	125
40	32	M	1.57	50	20.28	160	110	120	90	2	37.5	3.72	0.462	9.53	4.2	40.03	3.33	174	240	37	48	89
41	54	M	1.71	47	16.07	130	86	88	96	1.8	31.19	1.3	0.49	9.36	4.61	43.15	3.6	208	192	35	38.4	135
42	38	M	1.63	60	22.58	140	100	70	70	1.4	60.71	0.93	0.856	9.35	4.2	39.27	3.7	174	210	42	42	90
43	55	M	1.76	68	21.95	150	100	140	60	1.3	61.75	1.54	0.632	11.13	3.1	34.5	3.98	180	220	36	44	100
44	48	M	1.7	74	25.61	112	80	132	68	1.3	72.74	2.06	0.548	9.4	3.98	37.41	4.1	166	156	38	31.2	96.8
45	23	M	1.66	55	19.96	120	80	74	72	1.4	63.84	1.2	0.646	9.89	3.67	36.3	3.43	174	198	38	39.6	96.4
46	48	M	1.59	76	30.06	140	82	104	64	1.3	74.7	3.13	0.59	10.5	3.57	37.49	3.59	162	202	34	40.4	87.6
47	49	M	1.75	63	20.57	104	80	112	60	1.3	61.25	1.18	0.744	10.13	2.97	30.09	3.33	210	146	45	29.2	136
48	35	M	1.74	65	21.47	118	70	126	78	1.4	67.71	1.69	0.6	9.2	2.63	24.2	3.08	202	148	42	29.6	130
49	65	M	1.7	75	25.95	170	110	110	62	1.3	60.1	2.5	0.628	9.8	2.8	27.44	3.64	150	124	45	24.8	80.2
50	64	M	1.65	77	28.28	120	80	86	58	1.3	62.52	1.04	0.72	9.67	3.03	29.3	4.3	164	136	44	27.2	92.8
51	45	M	1.72	64	21.63	118	76	74	54	1.3	64.96	2.9	0.695	10.2	2.68	27.34	3.72	178	150	43	30	105
52	30	M	1.6	58	22.66	110	80	118	60	1.4	63.29	1.16	0.71	10.07	3.1	31.22	3.5	180	148	40	29.6	110
53	63	M	1.57	52	21.1	124	76	90	84	2.8	19.86	3.66	0.476	9.8	3.27	32.05	3.49	172	190	36	38	98
54	35	M	1.68	73	25.86	110	70	78	70	1.3	81.89	0.81	0.59	10.6	3.72	39.43	3.6	160	200	33	40	87
55	55	M	1.51	42	18.42	116	84	130	116	5.1	9.722	3.9	0.469	9.5	5.01	47.6	3.2	206	204	36	40.8	129
56	58	F	1.54	44	18.55	110	80	128	78	1.3	32.76	2.97	0.542	10.35	4.2	43.47	3.21	178	154	45	30.8	102
57	49	F	1.56	68	27.94	126	82	80	84	1.9	38.45	3.98	0.48	10.8	4.03	43.52	3.39	160	170	37	34	89

S.No	AGE	SEX	HT m	WT Kg	BMI Kg^2/m^2	SBP mmHg	DBP mmHg	FBG mg/dl	UREA mg/dl	CREAT mg/dl	Ccr ml/min	hsCRP mg/L	FETUIN-A g/L	CAL mg/dl	PHOS mg/dl	CaXP mg^2/dl^2	ALB g/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl
58	54	F	1.52	65	28.13	170	100	112	70	1.56	42.3	2.63	0.509	9.7	4.38	42.49	3.7	150	168	42	33.6	74.4
59	70	F	1.59	75	29.67	162	90	86	68	1.8	34.43	3.7	0.483	10.2	3.96	40.39	3.13	172	208	40	41.6	90.4
60	33	F	1.55	45	18.73	120	80	74	74	1.6	35.53	4.67	0.497	9.37	4.29	40.2	3.28	184	176	34	35.2	115
61	58	F	1.62	55	20.96	124	72	72	80	1.4	38.03	5.3	0.462	10.5	4.11	43.16	3.51	160	188	34	37.6	88.4
62	55	F	1.56	45	18.49	130	80	110	92	2.69	16.79	8.6	0.4	9.13	4.95	45.19	3.58	162	200	35	40	87
63	55	F	1.5	40	17.78	120	80	80	86	1.7	23.61	5	0.282	8.85	5.31	46.99	2.65	170	194	28	38.8	103
64	57	F	1.56	60	24.65	114	72	128	98	2.9	20.27	4.93	0.362	9.37	5.6	52.47	2.37	192	168	46	33.6	112
65	63	F	1.61	46	17.75	156	90	136	130	4.6	9.09	4.89	0.233	9.39	4.79	44.98	2.26	208	192	28	38.4	142
66	53	F	1.54	47	19.82	160	92	70	148	5.8	8.323	5.6	0.27	9.25	4.93	45.6	2.38	200	184	40	36.8	123
67	50	F	1.57	52	21.1	150	100	102	126	4.7	11.76	8.26	0.193	8.9	5.68	50.55	3.13	186	178	34	35.6	116
68	48	F	1.54	45	18.97	120	80	76	150	7.3	6.695	2.15	0.45	8.17	5.33	43.55	3.04	152	190	37	38	77
69	61	F	1.5	43	19.11	110	80	110	134	5.3	7.567	9.8	0.186	8.76	6.48	56.76	2.6	176	184	48	36.8	91.2
70	62	F	1.66	56	20.32	114	82	90	104	2.2	23.44	10.9	0.26	9.23	4.39	40.52	2.98	170	212	46	42.4	81.6
71	57	F	1.7	68	23.53	130	80	138	110	2.5	26.65	9.26	0.325	9.12	4.57	41.68	3.01	162	168	38	33.6	90.4
72	42	F	1.61	68	26.23	110	80	72	68	1.3	60.52	1.73	0.639	9.68	3.13	30.3	3.21	174	134	40	26.8	107
73	33	F	1.67	67	24.02	120	84	86	80	1.4	60.45	0.68	0.559	9.13	2.56	23.37	3.39	198	140	45	28	125
74	62	F	1.55	65	27.06	110	80	112	66	1.6	37.41	2.31	0.45	9.12	4.22	38.49	3.23	160	172	42	34.4	83.6
75	45	F	1.52	77	33.33	126	84	124	70	1.3	66.43	1.15	0.896	9.71	3.28	31.85	3.7	154	124	45	24.8	84.2
76	48	F	1.63	75	28.23	110	80	132	68	1.3	62.66	1.21	0.756	10.33	2.63	27.17	3.43	180	114	43	22.8	114
77	45	F	1.6	78	30.47	106	70	80	82	1.3	67.29	1.9	0.652	10.82	2.69	29.11	3.22	170	160	46	32	92
78	43	F	1.58	70	28.04	160	100	140	90	1.3	61.66	2.13	0.49	10.5	3	31.5	3.9	176	176	37	35.2	104
79	30	F	1.57	66	26.78	130	80	72	74	1.4	61.22	1.27	0.876	9.13	3.29	30.04	3.03	180	182	52	36.4	91.6
80	32	F	1.62	67	25.53	120	70	96	88	1.4	61.02	2.43	0.463	9.6	3.87	37.15	3.18	174	208	38	41.6	94.4

RESULTS

A total of 160 subjects were selected as the study group for the present study. This includes 80 cases with CKD and 80 healthy controls.

Levels of serum Fetuin-A, urea, creatinine, hsCRP, albumin, calcium, phosphorus, TC, TGL, HDL-C and FBG were estimated for all the samples of the study group. VLDL-C, LDL-C and Creatinine clearance were calculated from the formulas.

The values obtained in controls and cases are presented in the master chart I and II respectively.

Table 1 shows the baseline characteristics of the controls and cases.

Table 2 shows the gender-wise distribution of the study group. The distribution of males and females in the cases were 68.75% and 31.25% respectively, which shows the predominance of CKD in males.

Table 3 shows the age-wise distribution of the study group. The age group of cases ranged from 23-75 years, with the mean age of 50.4±11.89 years and median age of 50 years. The age group of controls ranged from 26-68 years, with the mean age of 47.85±10.55 years and median age of 46.5 years.

Table 4 shows the age and gender matched analysis of the study group. There is no significant difference between the age and gender of the two groups ($p=0.628$; >0.05 for controls; $p=0.812$; >0.05 for cases)

Table 5 shows the serum Fetuin-A levels in the study group. The mean value of serum Fetuin-A in cases was 0.4416±0.17 g/L and this was significantly lower than that of the control group (0.7527±0.18 g/L; p=0.001)

Table 6 shows the comparison of serum Fetuin-A levels in various age groups in the study subjects. Serum Fetuin-A levels were significantly lower in the cases than controls in all the included age groups.

Table 7 shows the gender-wise comparison of serum Fetuin-A in controls (Males: 0.7112±0.16 g/L; Females: 0.8441±0.19 g/L) and cases (Males: 0.4293±0.16 g/L; Females: 0.4686±0.19 g/L), which is highly significant (p=0.001).

Table 8 shows the gender-wise comparison of serum Fetuin-A levels within the cases (Males: 0.4293±0.16 g/L; Females: 0.4686±0.19 g/L). There is no significant difference of Fetuin-A levels between the genders in the cases (p=0.341; >0.05).

Table 9 shows the comparison of FBG, blood urea and creatinine and C_{Cr} in the study group. There is no significant difference in FBG between the two groups (p=0.122; > 0.05). Blood urea was found to be significantly higher and serum creatinine and C_{Cr} were significantly lower in the cases than controls (p=0.001).

Table 10 shows the comparison of serum hsCRP and albumin levels in the study group. hsCRP levels were significantly higher and albumin levels were significantly lower in the cases than controls (p=0.001).

Table 11 shows the comparison of serum calcium, phosphorus and calcium-phosphorus product in the study group. Serum calcium levels were found to be significantly lower and the serum phosphorus and Ca X P were significantly higher in the cases than controls (p=0.001).

Table 12 shows the comparison of lipid parameters in the study group. We observed significantly higher serum TGL and VLDL-C levels and significantly lower HDL-C levels in the cases than controls (p< 0.0001). There is no significant difference in the TC and LDL-C levels between the two groups (p=0.192; >0.05).

Table 13 shows the comparison of serum Fetuin-A levels in the study group in relation to C_{Cr}. As the renal function declined, we observed a progressive reduction in the Fetuin-A levels in cases when compared to controls.

Table 14 shows the comparison of serum hsCRP levels in the study group in relation to C_{Cr}. We observed a progressive increase in the hsCRP values in cases as the renal function declined when compared to controls.

Table 15 shows the comparison of blood urea levels in the study group in relation to C_{Cr}. There is a progressive increase in the urea values in cases with lessening renal function as compared to controls.

Table 16 shows the comparison of serum creatinine levels in the study group in relation to C_{Cr}. We observed a progressive reduction of creatinine values as the renal function decreased in cases. Serum creatinine levels were within the normal reference range in the controls.

Table 17 shows comparison of serum calcium levels in the study group in relation to C_{Cr}. There is a serial reduction in the calcium levels with declining renal function in cases. Serum calcium levels were within the normal reference range in the controls.

Table 18 shows the comparison of serum phosphorus levels in the study group in relation to C_{Cr}. We observed a progressive increase in the phosphorus levels in the cases as the renal function declined. Serum phosphorus levels were within the normal reference range in the controls.

Table 19 shows the comparison of Ca X P levels in the study group in relation to C_{Cr}. There is a progressive increase in the Ca X P values in cases with lessening renal function. Ca X P was within the normal reference range in the controls.

Table 20 shows the comparison of serum albumin levels in the study group in relation to C_{Cr}. We observed a progressive reduction of albumin levels as the renal function decreased in the cases. Serum albumin levels were within the normal reference range in the controls.

Table 21 shows the comparison of lipid parameters in the study group in relation to C_{Cr}. Serum TC and LDL-C levels were found to be within the normal reference range in the cases irrespective of C_{Cr}. We observed a serial increase in the TGL and VLDL-C levels and a serial decrease in the HDL-C levels with lessening renal function.

Table 22 shows the Pearson's coefficient of correlation between serum Fetuin-A and the other studied biochemical parameters in the cases. There is a highly significant negative correlation of Fetuin-A with serum urea, creatinine, hsCRP, phosphorus, Ca X P, TGL and VLDL-C ($p < 0.01$). We also observed a highly significant positive correlation of Fetuin-A with C_{Cr}, albumin, calcium and HDL-C.

Figure 13: Bar diagram showing the serum Fetuin-A levels in the study group.

Figure14: Bar diagram showing the comparison of serum hsCRP and albumin levels in the study group.

Figure15: Bar diagram showing the comparison of serum calcium, phosphorus and calcium-phosphorus product in the study group.

Figure 16: Bar diagram showing the comparison of serum Fetuin-A levels in the study group in relation to creatinine clearance.

Figure 17: Bar diagram showing the comparison of serum hsCRP levels in the study group in relation to creatinine clearance.

Figure 18: Bar diagram showing the comparison of serum calcium-phosphorus product in the study group in relation to creatinine clearance.

Figure 19: Scatter diagram of serum Fetuin-A vs hsCRP in controls

Figure 20: Scatter diagram of serum Fetuin-A vs hsCRP in cases

Figure 21: Scatter diagram of serum Fetuin-A vs albumin in controls

STATISTICAL ANALYSIS

- Student’s t-test and Chi-square test were employed for the statistical analysis of data.
- The data were expressed in terms of mean and standard deviation.
- ‘P’ value less than 0.05 was taken as the significant value.
- Correlation between the measured parameters was assessed using Pearson’s correlation coefficient.

TABLE 1

DESCRIPTIVE STATISTICS OF THE STUDY GROUP

S.NO	PARAMETERS	CONTROLS (n=80)				CASES (n=80)			
		MIN.	MAX.	MEAN	S.D	MIN.	MAX.	MEAN	S.D
1	**AGE (years)**	26	68	47.85	10.552	23	75	50.40	11.895
2	**HEIGHT (m)**	1.50	1.72	1.603	0.0591	1.45	1.78	1.6146	.0723
3	**WEIGHT (kg)**	47	76	63.19	5.934	35	86	57.33	11.775
4	**BMI (kg/m^2)**	18.591	31.111	24.561	2.700	14.951	33.327	21.942	4.163
5	**SBP (mmHg)**	88	130	106.70	10.636	90	180	128.32	19.721
6	**DBP (mmHg)**	70	86	74.97	4.173	60	110	83.03	10.208
7	**S.FETUIN-A (g/L)**	0.51	1.23	0.752	0.176	0.15	0.90	0.4416	0.170
8	**FBG (mg/dl)**	72	104	91.05	9.657	70	148	103.67	23.098
9	**B.UREA (mg/dl)**	18	28	22.92	3.244	54	160	99.95	27.731
10	**S.CREAT (mg/dl)**	0.70	1.10	0.841	0.082	1.30	9.60	3.068	2.153
11	**Ccr (ml/min)**	79.166	134.58	96.476	8.409	5.786	81.891	32.994	21.241
12	**S.hsCRP (mg/L)**	0.21	0.98	0.568	0.232	0.68	10.90	4.618	3.037
13	**S.CALCIUM (mg/dl)**	9.12	10.90	9.905	0.497	8.17	11.13	9.542	0.675
14	**S.PHOS (mg/dl)**	2.67	4.30	3.410	0.367	2.56	7.00	4.539	1.105
15	**Ca X P (mg^2/dl^2)**	25.867	39.900	33.711	3.285	23.372	59.710	42.844	8.718
16	**S.ALBUMIN (g/dl)**	3.56	4.89	4.005	0.332	2.13	4.30	3.208	0.432
17	**S.TC (mg/dl)**	160	208	173.575	14.501	150	220	175.375	16.343
18	**S.TGL (mg/dl)**	100	168	133.96	16.363	114	240	182.50	26.576
19	**S.HDL-C (mg/dl)**	35	50	41.72	3.368	26	52	38.03	5.202
20	**S.VLDL-C (mg/dl)**	20.00	33.60	26.792	3.272	22.80	48.00	36.500	5.315
21	**S.LDL-C (mg/dl)**	84.00	142.60	105.057	14.74	61.00	150.40	101.065	18.089

TABLE 2

GENDERWISE DISTRIBUTION OF THE STUDY GROUP

GROUPS	GENDER	
	MALE (%)	FEMALE (%)
CONTROLS (n=80)	55 (68.75%)	25 (31.25%)
CASES (n=80)	55 (68.75%)	25 (31.25%)

TABLE 3

AGEWISE DISTRIBUTION OF THE STUDY GROUP

GROUPS	AGE (YEARS)		MEDIAN AGE (YEARS)	RANGE (YEARS)
	MEAN	SD		
CONTROLS (n=80)	47.85	10.552	46.5	26-68
CASES (n=80)	50.40	11.895	50	23-75

TABLE 4

AGE AND GENDER MATCHED ANALYSIS OF THE STUDY GROUP

S.No	Age	Gender					
		Controls			Cases		
		Male (n=55)	Female (n=25)	Statistical Inference	Male (n=55)	Female (n=25)	Statistical Inference
1	Below 30yrs	2 (3.6%)	1 (4%)	χ^2 =2.592 P=0.628 Not Significant	2 (3.6%)	1 (4%)	χ^2 = 1.582 P=0.812 Not significant
2	31 to 40yrs	14 (25.5%)	3 (12%)		12 (12.8%)	3 (12%)	
3	41 to 50yrs	15 (27.3%)	10 (40%)		15 (27.3%)	8 (32%)	
4	51 to 60 years	11 (20%)	6 (24%)		13 (23.6%)	8 (32%)	
5	>60 years	13 (23.6%)	5 (20%)		13 (23.6%)	5 (20%)	

TABLE 5

COMPARISON OF SERUM FETUIN-A IN THE STUDY GROUP

S.NO	GROUPS	S.FETUIN-A (g/L)		STATISTICAL INFERENCE
		MEAN	SD	
1	CONTROLS (n=80)	0.7527	0.17686	T=11.344
2	CASES (n=80)	0.4416	0.17002	P<0.001 Significant

Figure 13

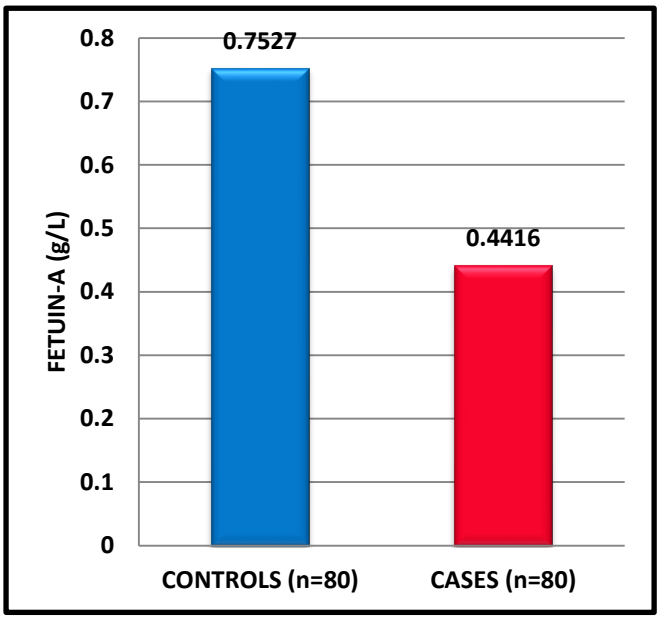


TABLE 6

AGE MATCHED ANALYSIS OF SERUM FETUIN-A LEVELS IN THE STUDY GROUP

AGE	S.FETUIN-A (g/L)			STATISTICAL INFERENCE
	GROUPS	MEAN	SD	
< 30 yrs	CONTROLS (n=3)	0.7390	0.22111	T = 8.135 P=0.007 Significant
	CASES (n=3)	0.6440	0.11871	
31-40 yrs	CONTROLS (n=17)	0.7532	0.19389	T = 4.639 P<0.001 Significant
	CASES (n=15)	0.4648	0.15180	
41-50 yrs	CONTROLS (n=25)	0.7932	0.16947	T = 5.902 P<0.001 Significant
	CASES (n=23)	0.4893	0.18718	
51-60 yrs	CONTROLS (n=17)	0.7598	0.18164	T = 7.484 P<0.001 Significant
	CASES (n=21)	0.3936	0.11879	
>60 yrs	CONTROLS (n=18)	0.6916	0.16181	T = 6.200 P<0.001 Significant
	CASES (n=18)	0.3667	0.15254	

TABLE 7

GENDER MATCHED COMPARISON OF SERUM FETUIN-A LEVELS IN THE STUDY GROUP

GENDER	GROUPS	S.FETUIN-A (g/L)		STATISTICAL INFERENCE
		MEAN	SD	
MALE	CONTROLS (n=55)	0.7112	0.15636	T=9.350 P<0.001 Significant
	CASES (n=55)	0.4293	0.15985	
FEMALE	CONTROLS (n=25)	0.8441	0.18790	T=7.004 P<0.001 Significant
	CASES (n=25)	0.4686	0.19119	

TABLE 8

GENDERWISE DIFFERENCE OF SERUM FETUIN-A LEVEL IN CASES

.NO	GENDER	S.FETUIN-A (g/L)		STATISTICAL INFERENCE
		MEAN	SD	
1	MALE (n=55)	0.4293	0.15985	T = -0.959 P=0.341 Not Significant
2	FEMALE (n=25)	0.4686	0.19119	

TABLE 9

COMPARISON OF FBG, BLOOD UREA, SERUM CREATININE AND CREATININE CLEARANCE (C_{cr}) IN THE STUDY GROUP

PARAMETERS	GROUPS	MEAN	SD	STATISTICAL INFERENCE
FBG (mg/dl)	CONTROLS (n=80)	91.05	9.65	T=12.307 P=0.102 Not Significant
	CASES (n=80)	103.68	23.09	
B.UREA (mg/dl)	CONTROLS (n=80)	22.93	3.24	T=-24.675 P<0.001 Significant
	CASES (n=80)	99.95	27.73	
S.CREATININE (mg/dl)	CONTROLS (n=80)	0.84	0.08	T=-9.243 P<0.001 Significant
	CASES (n=80)	3.068	2.15	
C_{cr} (ml/min)	CONTROLS (n=80)	96.47	8.40	T=24.854 P<0.001 Significant
	CASES (n=80)	32.99	21.24	

TABLE 10

COMPARISON OF SERUM hsCRP AND ALBUMIN IN THE STUDY GROUP

PARAMETERS	GROUPS	MEAN	SD	STATISTICAL INFERENCE
S.hsCRP (mg/L)	CONTROLS (n=80)	0.5681	0.2327	T=-11.893 P<0.001 Significant
	CASES (n=80)	4.6189	3.0376	
S.ALBUMIN (g/dl)	CONTROLS (n=80)	4.0055	0.3326	T=13.060 P<0.001 Significant
	CASES (n=80)	3.2087	0.4325	

Figure 14

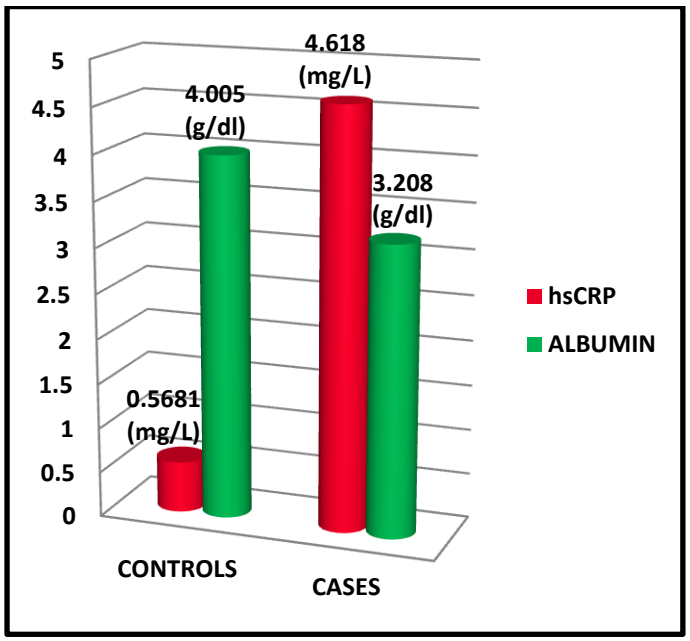


TABLE 11

COMPARISON OF SERUM CALCIUM, PHOSPHORUS AND CALCIUM-PHOSPHORUS PRODUCT IN THE STUDY GROUP

PARAMETERS	GROUPS	MEAN	S.D	STATISTICAL INFERENCE
S.CALCIUM (mg/dl)	CONTROLS (n=80)	9.9058	0.4971	T=3.273 P<0.001 Significant
	CASES (n=80)	9.5426	0.6754	
S.PHOSPHORUS (mg/dl)	CONTROLS (n=80)	3.4105	0.3676	T=-8.667 P<0.001 Significant
	CASES (n=80)	4.5394	1.1054	
Ca X P (mg^2/dl^2)	CONTROLS (n=80)	33.7118	3.2850	T=-8.768 P<0.001 Significant
	CASES (n=80)	42.8447	8.7187	

Figure 15

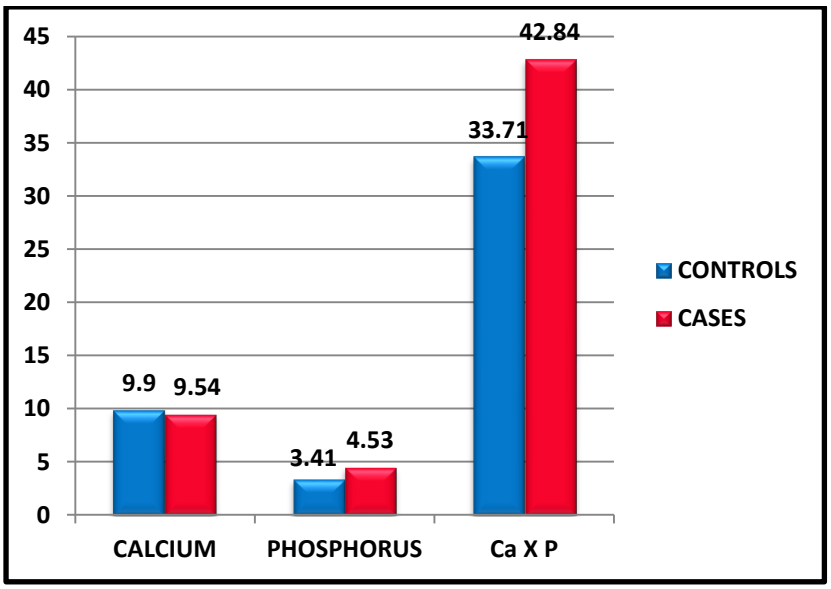


TABLE 12

COMPARISON OF LIPID PARAMETERS IN THE STUDY GROUP

PARAMETERS	GROUPS	MEAN	S.D	STATISTICAL INFERENCE
S.TC (mg/dl)	CONTROLS (n=80)	173.57	14.74	T=1.309 P=0.462 Not Significant
	CASES (n=80)	175.37	18.08	
S.TGL (mg/dl)	CONTROLS (n=80)	133.96	16.36	T=-13.910 P<0.001 Significant
	CASES (n=80)	182.50	26.57	
S.HDL-C (mg/dl)	CONTROLS (n=80)	41.73	3.36	T=5.340 P<0.001 Significant
	CASES (n=80)	38.03	5.202	
S.VLDL-C (mg/dl)	CONTROLS (n=80)	26.79	3.27	T=-13.910 P<0.001 Significant
	CASES (n=80)	36.50	5.31	
S.LDL-C (mg/dl)	CONTROLS (n=80)	105.05	14.74	T=3.528 P<0.128 Not Significant
	CASES (n=80)	101.06	18.08	

TABLE 13

COMPARISON OF SERUM FETUIN-A IN THE STUDY GROUP IN RELATION TO CREATININE CLEARANCE

GROUPS	S.FETUIN-A (g/L)	
	MEAN	SD
C_{cr} (ml/min)		
60-90 (n=20)	0.6645	0.1193
30-59 (n=20)	0.4684	0.0626
15-29 (n=20)	0.3648	0.0554
< 15 (n=20)	0.2685	0.0905
TOTAL CASES (n=80)	0.4416	0.17
CONTROLS (n=80)	0.7527	0.1768

Figure 16

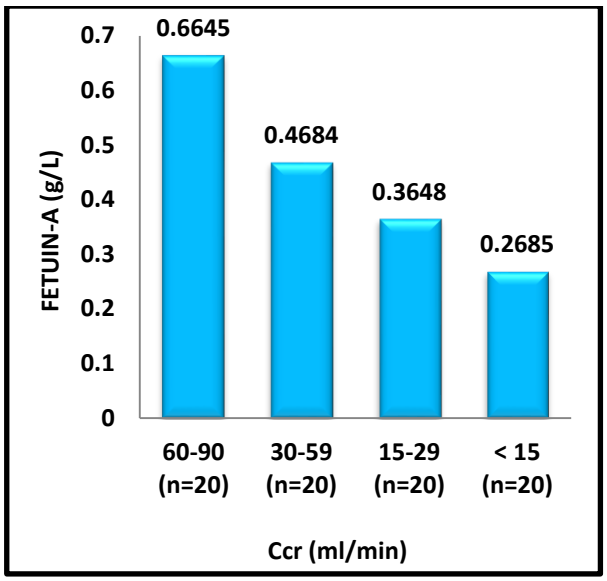


TABLE 14

COMPARISON OF SERUM hsCRP IN THE STUDY GROUP IN RELATION TO CREATININE CLEARANCE

GROUPS	S.hsCRP (mg/L)	
	MEAN	SD
C_{cr} (ml/min)		
60 to 90 (n=20)	1.632	0.7014
30 to 59 (n=20)	3.0795	1.1962
15 to 29 (n=20)	6.206	2.6283
< 15 (n=20)	7.558	2.4301
TOTAL CASES (n=80)	4.6189	3.0376
CONTROLS (n=80)	0.5681	0.2327

Figure 17

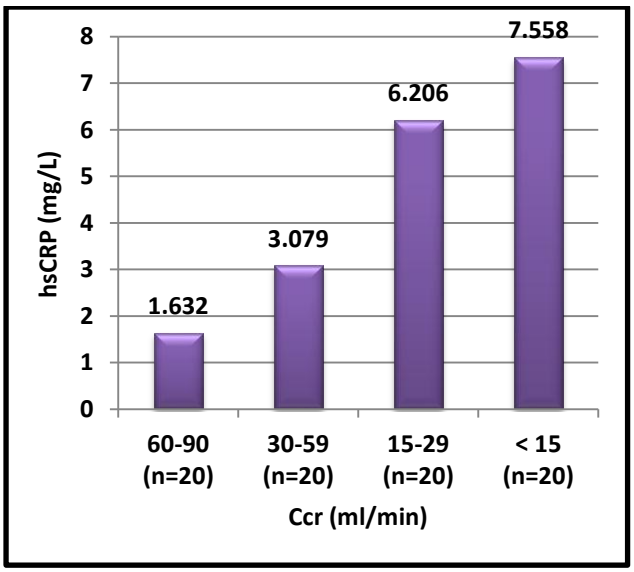


TABLE 15: COMPARISON OF BLOOD UREA IN THE STUDY GROUP IN RELATION TO CREATININE CLEARANCE

GROUPS	B.UREA (mg/dl)	
	MEAN	SD
C_{cr} (ml/min)		
60 to 90 (n=20)	69.8	9.903
30 to 59 (n=20)	89.1	15.553
15 to 29 (n=20)	110.6	18.785
< 15 (n=20)	130.3	18.299
TOTALCASES (n=80)	99.95	27.731
CONTROLS (n=80)	22.93	3.244

TABLE 16: COMPARISON OF SERUM CREATININE IN THE STUDY GROUP IN RELATION TO CREATININE CLEARANCE

GROUPS	S.CREAT (mg/dl)	
	MEAN	SD
C_{cr} (ml/min)		
60 to 90 (n=20)	1.335	0.0489
30 to 59 (n=20)	1.859	0.3061
15 to 29 (n=20)	3.1645	1.0203
< 15 (n=20)	5.9145	2.2151
TOTAL CASES (n=80)	3.0683	2.15350
CONTROLS (n=80)	0.8412	0.08221

TABLE 17

COMPARISON OF SERUM CALCIUM IN THE STUDY GROUP IN RELATION TO CREATININE CLEARANCE

GROUPS	S.CALCIUM (mg/dl)	
	MEAN	SD
C_{cr} (ml/min)		
60 to 90 (n=20)	9.942	0.5784
30 to 59 (n=20)	9.868	0.5682
15 to 29 (n=20)	9.328	0.5608
< 15 (n=20)	8.9325	0.3555
TOTAL CASES (n=80)	9.5426	0.6754
CONTROLS (n=80)	9.9058	0.49711

TABLE 18

COMPARISON OF SERUM PHOSPHORUS IN THE STUDY GROUP IN RELATION TO CREATININE CLEARANCE

GROUPS	S.PHOSPHORUS (mg/dl)	
	MEAN	SD
C_{cr} (ml/min)		
60 to 90 (n=20)	3.195	0.4918
30 to 59 (n=20)	4.1635	0.2607
15 to 29 (n=20)	4.916	0.5384
< 15 (n=20)	5.883	0.6171
TOTAL CASES (n=80)	4.5394	1.1054
CONTROLS (n=80)	3.4105	0.3676

TABLE 19

COMPARISON OF SERUM CALCIUM-PHOSPHORUS PRODUCT IN THE STUDY GROUP IN RELATION TO CREATININE CLEARANCE

GROUPS	Ca X P (mg^2/dl^2)	
	MEAN	SD
C_{cr} (ml/min)		
60 to 90 (n=20)	31.723	4.8416
30 to 59 (n=20)	41.466	3.0865
15 to 29 (n=20)	45.765	4.9648
< 15 (n=20)	52.425	4.5444
TOTAL CASES (n=80)	42.844	8.718
CONTROLS (n=80)	33.711	3.285

Figure 18

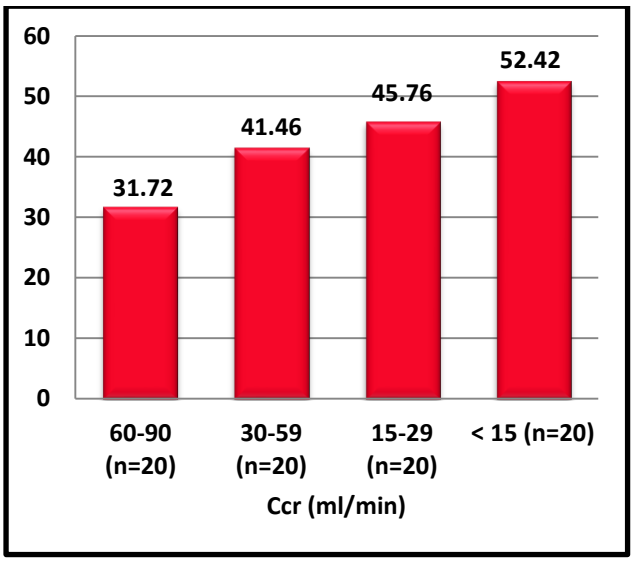


TABLE 20

COMPARISON OF SERUM ALBUMIN IN THE STUDY GROUP IN RELATION TO CREATININE CLEARANCE

GROUPS	S.ALBUMIN (g/dl)	
	MEAN	SD
C_{cr} (ml/min)		
60 to 90 (n=20)	3.5515	0.3416
30 to 59 (n=20)	3.3465	0.2785
15 to 29 (n=20)	3.1	0.3483
< 15 (n=20)	2.837	0.4011
TOTAL CASES (n=80)	3.2087	0.4325
CONTROLS (n=80)	4.0055	0.3326

TABLE 21

COMPARISON OF LIPID PARAMETERS IN CASES IN RELATION TO CREATININE CLEARANCE

PARAMETERS	C_{cr} (ml/min)			
	60-90 (n=20)	**30-50 (n=20)**	**15-29 (n=20)**	**<15 (n=20)**
S.TC (mg/dl)				
MEAN	179.60	176.40	173.30	187.60
SD	15.756	15.608	11.131	19.699
S.TGL (mg/dl)				
MEAN	164.30	187.20	188.35	190.15
SD	33.594	22.816	17.160	22.816
S.HDL-C (mg/dl)				
MEAN	41.30	37.60	37.40	35.80
SD	4.646	3.560	5.103	5.926
S.VLDL-C (mg/dl)				
MEAN	32.86	37.44	37.67	38.03
SD	6.7187	4.5632	4.5765	3.4319
S.LDL-C (mg/dl)				
MEAN	105.44	101.36	97.87	114.13
SD	16.7605	19.9041	13.3826	21.9760

TABLE 22

PEARSON'S CORRELATION COEFFICIENT BETWEEN S.FETUIN-A AND OTHER BIOCHEMICAL PARAMETERS IN CASES (n=80)

PARAMETERS	CORRELATION VALUE	STATISTICAL INFERENCE
B.UREA (mg/dl)	-.691(**)	P < 0.01 Significant
S.CREATININE (mg/dl)	-.595(**)	P < 0.01 Significant
C_{cr} (ml/min)	.855(**)	P < 0.01 Significant
S.hsCRP (mg/L)	-.756(**)	P < 0.01 Significant
S.CALCIUM (mg/dl)	.464(**)	P < 0.01 Significant
S.PHOSPHORUS (mg/dl)	-.819(**)	P < 0.01 Significant
Ca X P (mg^2/dl^2)	-.818(**)	P < 0.01 Significant
S.ALBUMIN (g/dl)	.616(**)	P < 0.01 Significant
S.TC (mg/dl)	-.078	P > 0.05 Not Significant
S.TGL (mg/dl)	-.366(**)	P < 0.01 Significant
S.HDL-C (mg/dl)	.443(**)	P < 0.01 Significant
S.VLDL-C (mg/dl)	-.366(**)	P < 0.01 Significant
S.LDL-C (mg/dl)	-.086	P > 0.05 Not Significant

** Correlation is significant at P<0.01 level

Figure 19: SCATTER DIAGRAM OF SERUM FETUIN-A VS hsCRP IN CONTROLS

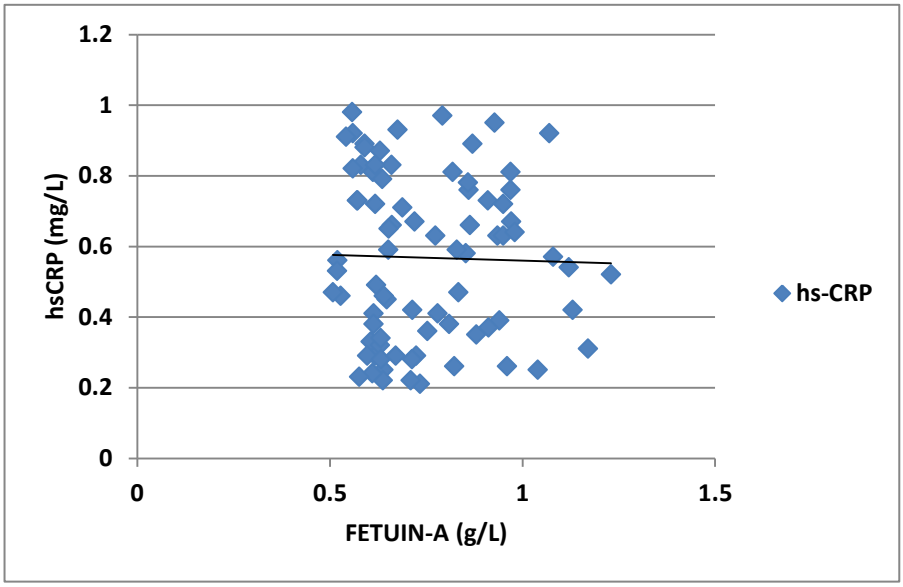


Figure 20: SCATTER DIAGRAM OF SERUM FETUIN-A VS hsCRP IN CASES

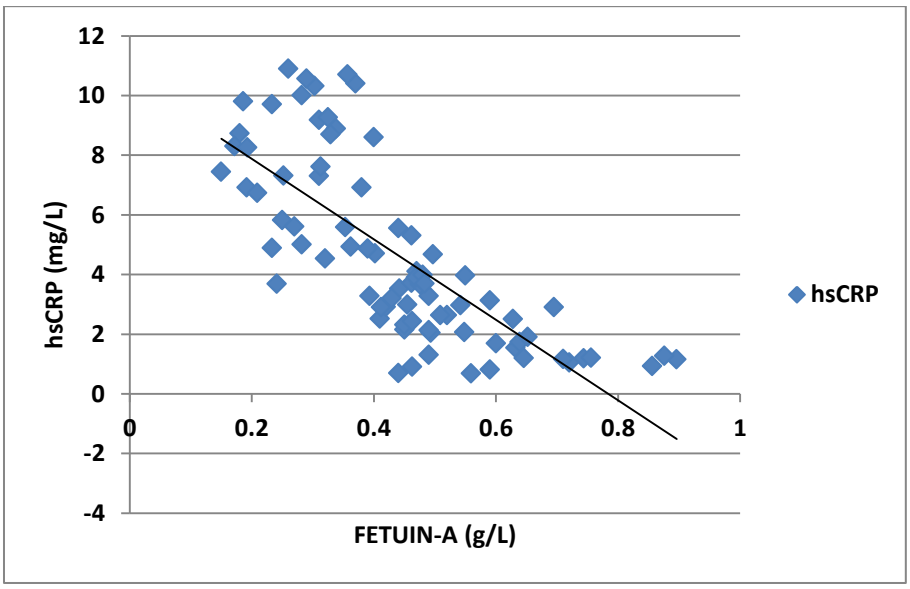


Figure 21: SCATTER DIAGRAM OF SERUM FETUIN-A VS ALBUMIN IN CONTROLS

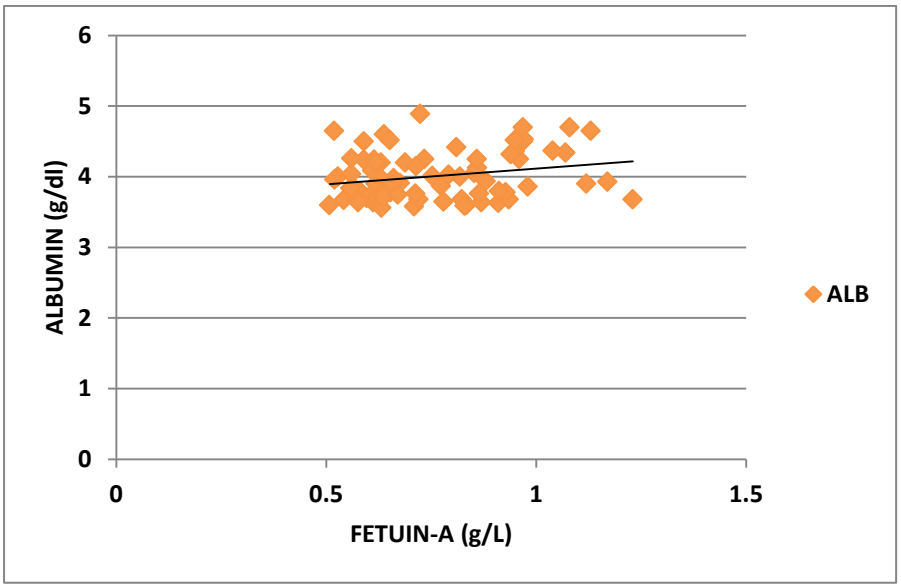


Figure 22: SCATTER DIAGRAM OF SERUM FETUIN-A VS ALBUMIN IN CASES

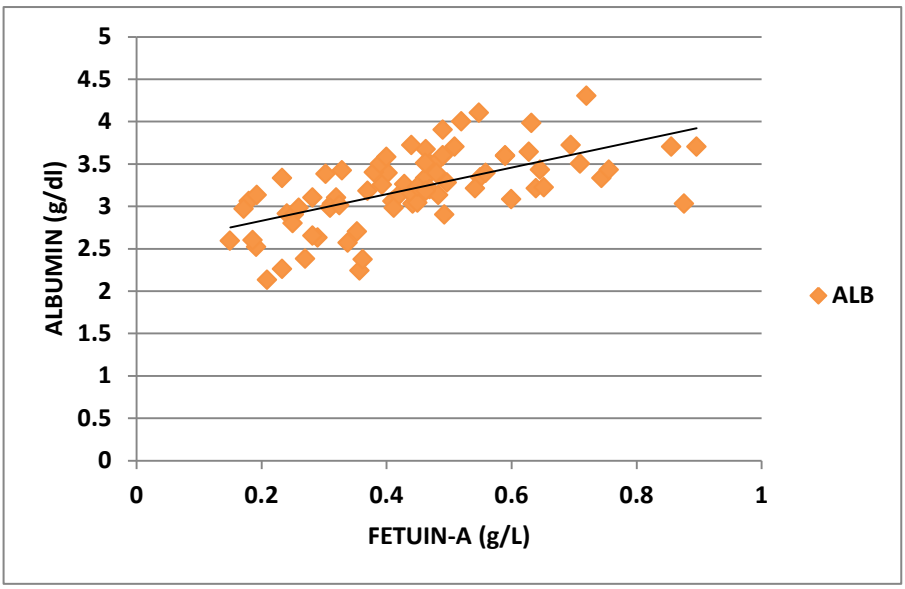


Figure 23: SCATTER DIAGRAM OF SERUM FETUIN-A VS PHOSPHORUS IN CONTROLS

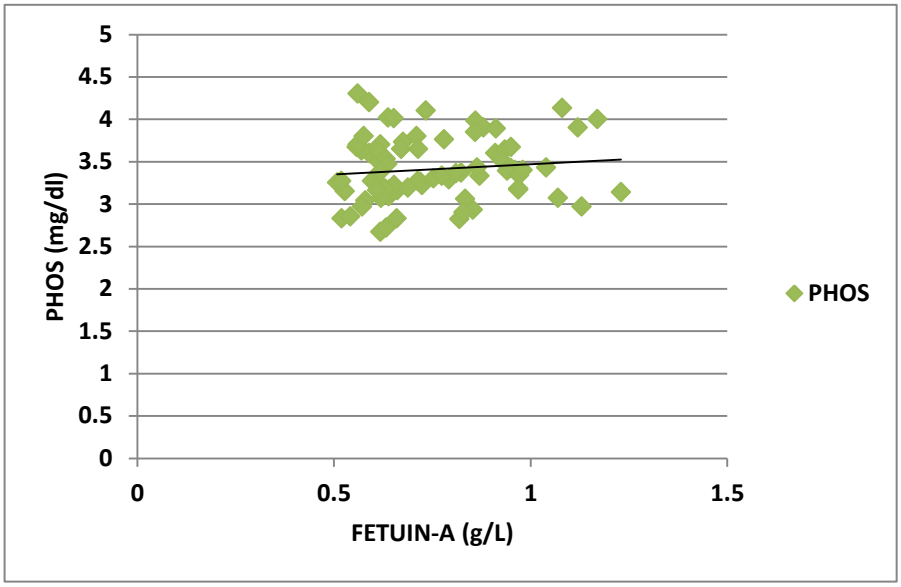


Figure 24: SCATTER DIAGRAM OF SERUM FETUIN-A VS PHOSPHORUS IN CASES

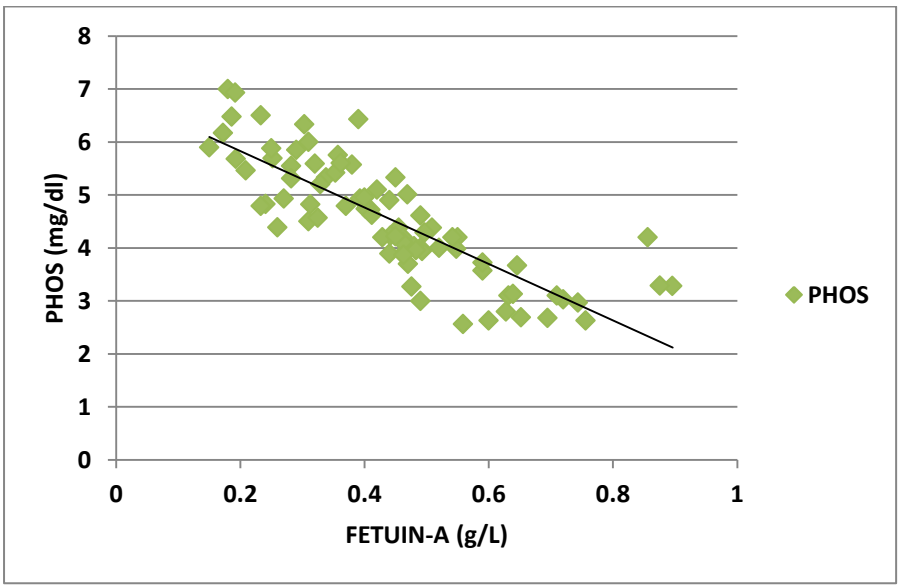


Figure 25: SCATTER DIAGRAM OF SERUM FETUIN-A VS CALCIUM-PHOSPHORUS PRODUCT IN CONTROLS

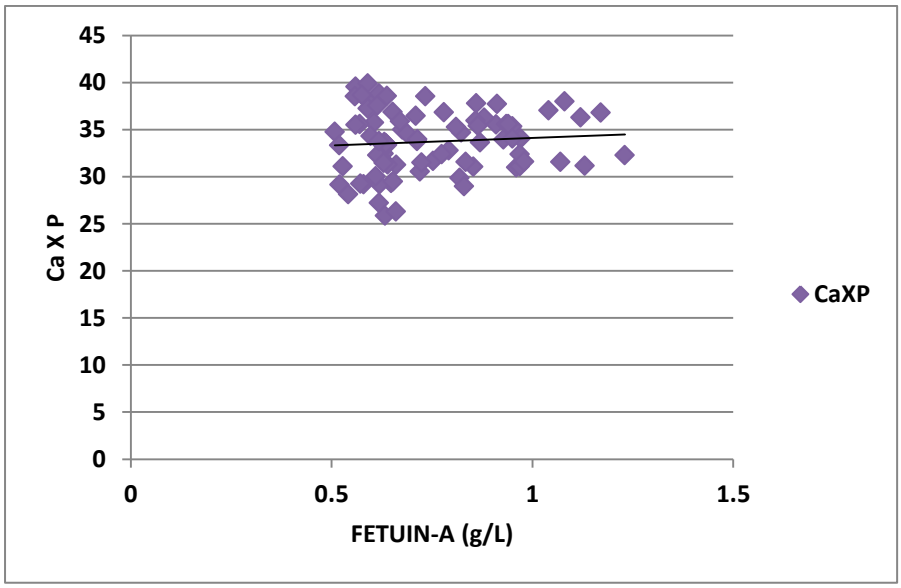


Figure 26: SCATTER DIAGRAM OF SERUM FETUIN-A VS CALCIUM-PHOSPHORUS PRODUCT IN CASES

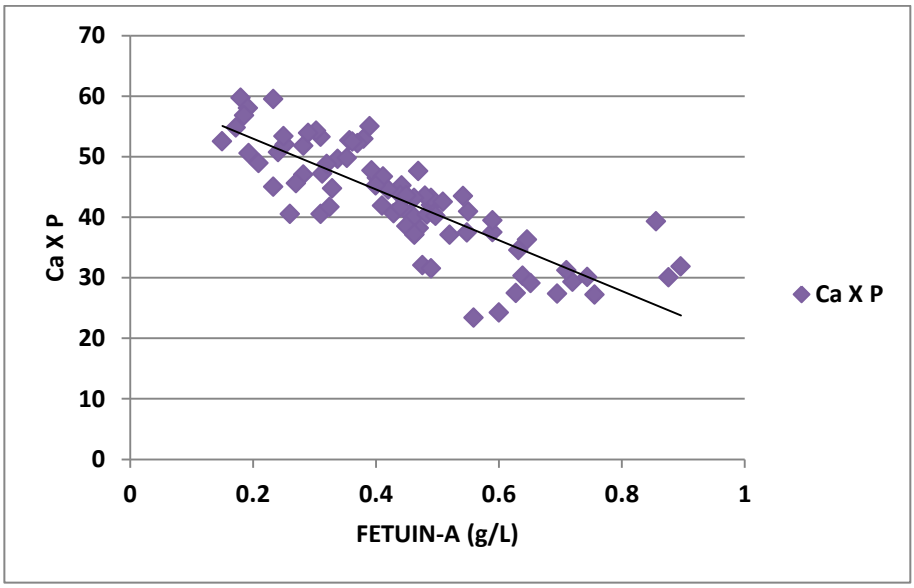


Figure 27: SCATTER DIAGRAM OF SERUM FETUIN-A VS CREATININE CLEARANCE IN CONTROLS

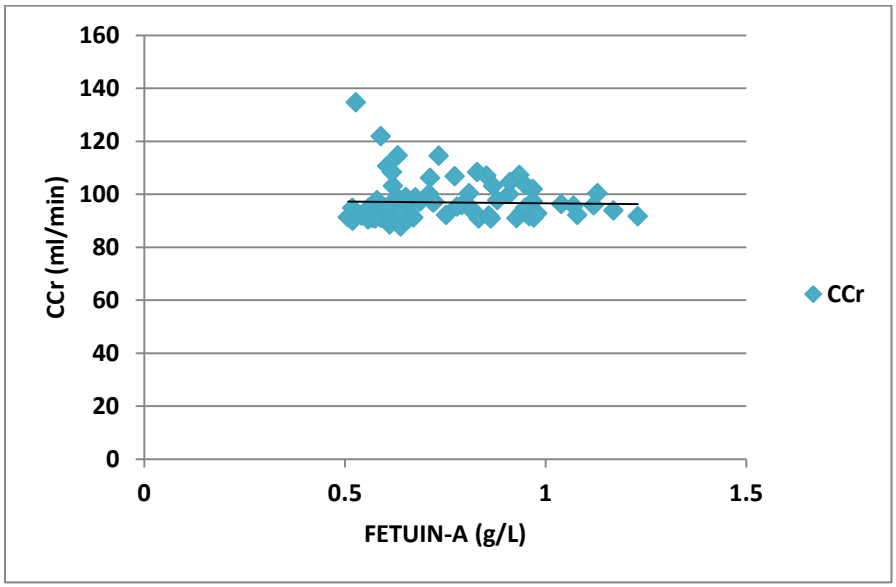


Figure 28: SCATTER DIAGRAM OF SERUM FETUIN-A VS CREATININE CLEARANCE IN CASES

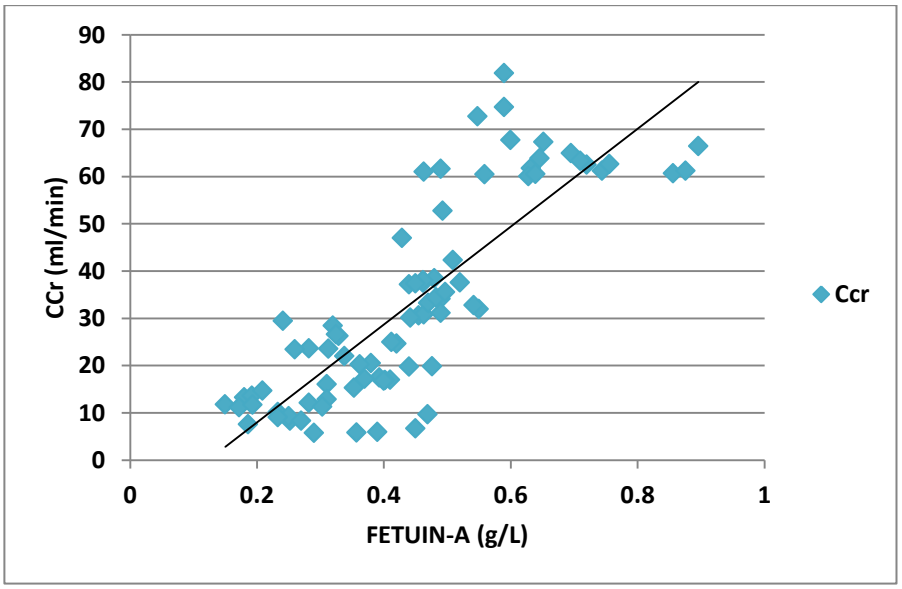


Figure 29: SCATTER DIAGRAM OF SERUM FETUIN-A VS TGL IN CONTROLS

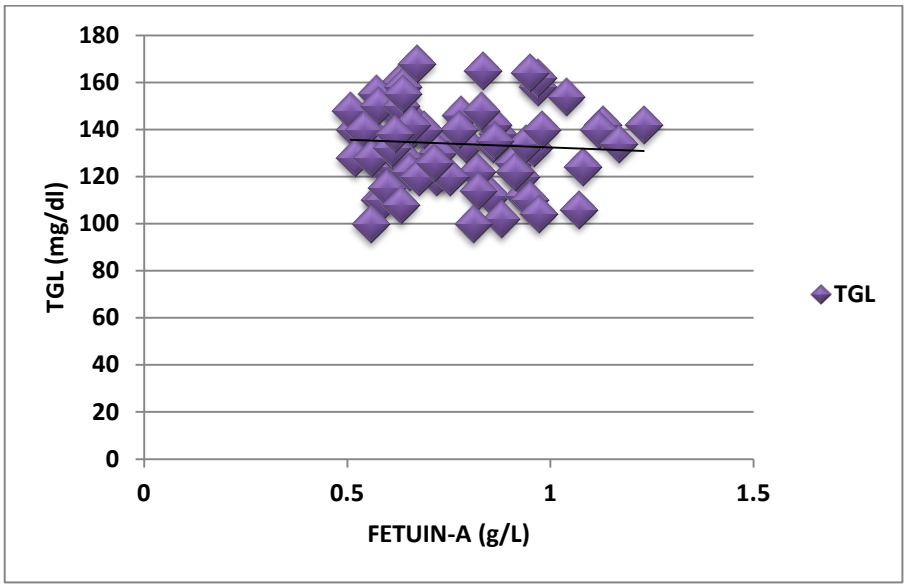


Figure 30: SCATTER DIAGRAM OF SERUM FETUIN-A VS TGL IN CASES

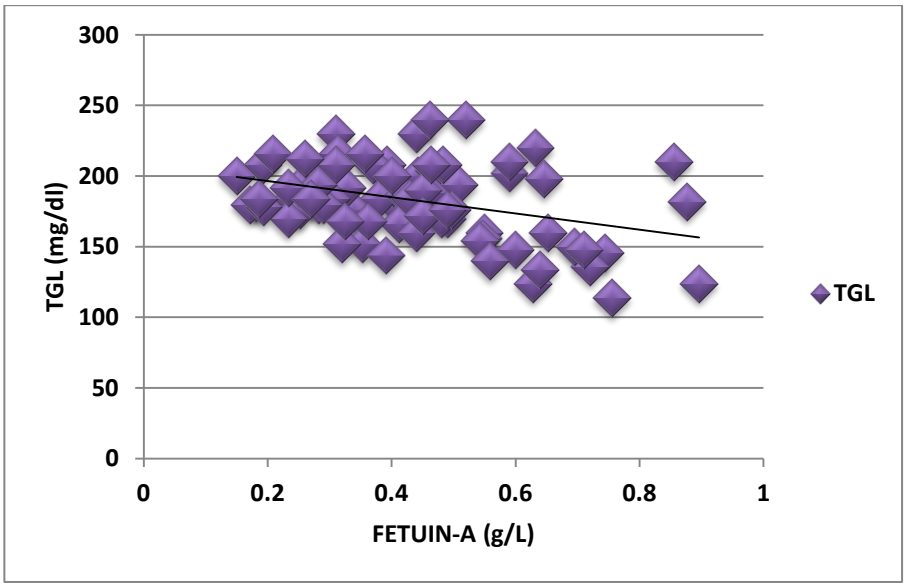


Figure 31: SCATTER DIAGRAM OF SERUM FETUIN-A VS HDL IN CONTROLS

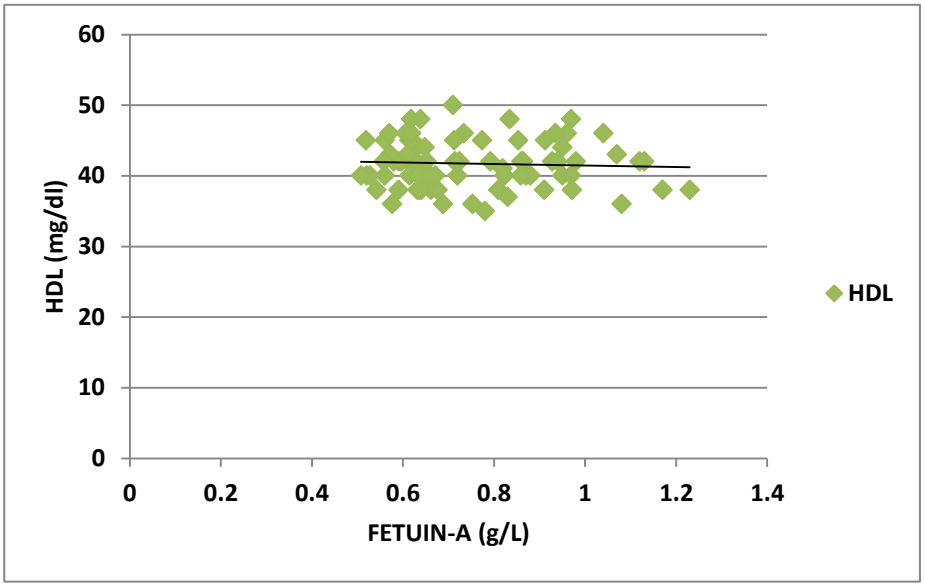
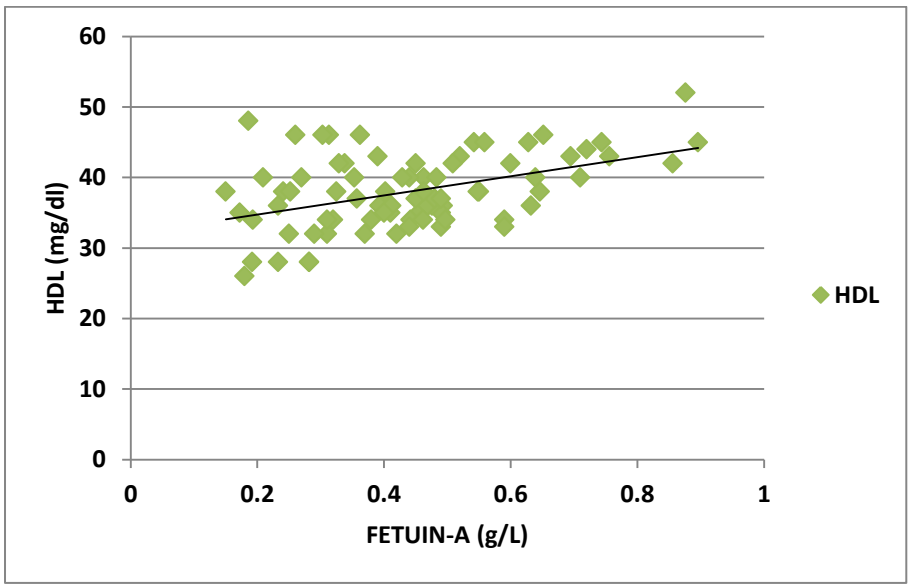


Figure 32: SCATTER DIAGRAM OF SERUM FETUIN-A VS HDL IN CASES



DISCUSSION

In the present study, serum Fetuin-A concentrations were found to be significantly decreased in patients with CKD [Mean value: 0.4416±0.17 g/L] when compared to the control group [Mean value: 0.7527±0.18 g/L; p=0.001].

When patients in different stages of CKD were compared, Fetuin-A levels were found to be progressively decreased from stage 2 (C_{Cr}=60-90 ml/min) to stage 5 (C_{Cr}<15 ml/min), in comparison with the control group. This observation shows that reduction in serum Fetuin-A levels develop relatively in the early stages of CKD. This finding is supported further by the highly significant negative correlation observed between serum Fetuin-A and creatinine in CKD cases (r=-0.595, p<0.01).

These findings are in accordance with the study of Cagler et al., which reported a decrease in serum Fetuin-A levels in all stages of CKD, except stage 1[75]. Lower levels of serum Fetuin-A were also reported in hemodialysis patients in the previous studies[76,77].

The mean level of serum Fetuin-A in CKD cases in the present study is 0.4416±0.17 g/L. This finding is almost consistent with that of Cottone et al., where the mean Fetuin-A concentration was 0.53±0.17 g/L in patients with CKD[78]. We also observed that serum Fetuin-A levels were significantly lower in all age groups and in both genders when compared to controls, which

indicates that age and gender does not have an impact on serum Fetuin-A levels.

CKD is a state of chronic persistent low-grade inflammation in which there is a chronic systemic elevation of pro-inflammatory markers. The prototypic marker of inflammation in the clinical setting is hsCRP, a positive acute phase reactant and higher level of this inflammatory biomarker is associated with cardiovascular mortality in patients with renal insufficiency[79,80].

In the present study, we observed significantly higher levels of hsCRP in CKD cases when compared to controls [Mean level: Cases- 4.618±3.03 mg/L; Controls- 0.567±0.23 mg/L; p=0.001]. As the renal function declined, we observed a progressive increase in the hsCRP levels. Further a strong inverse correlation was found between Fetuin-A and hsCRP levels (r=-0.756; $p<0.01$) which shows that Fetuin-A is a negative acute phase reactant.

Serum albumin is regarded as one of the negative acute phase reactant and a low serum albumin level in CKD is a well established predictor of mortality[81]. In the present study, serum albumin was found to be significantly reduced in CKD cases [Mean level: Cases-3.2±0.43g/dl; Controls-4.005±0.33g/dl; p=0.001]. We also observed a progressive decrease in the serum albumin levels with declining renal function. The results also revealed a strong positive correlation of serum Fetuin-A with albumin levels (r=0.616; $p<0.01$).

These results of the present study are in accordance with the previous studies[82,91], suggesting a global pro-atherogenic inflammatory activation even in early stages of CKD leading onto down regulation of serum Fetuin-A.

Hyperphosphatemia due to phosphorus retention is associated with increased mortality in individuals with moderate to severe CKD[83]. In the present study, the serum phosphorus levels were found to be significantly elevated in CKD cases [Mean level: Cases- 4.54±1.1mg/dl; Controls- 3.41±0.37mg/dl; p=0.001]. The results also showed a progressive increase in the phosphorus levels as the renal function declined. We also found a strong significant negative correlation of serum Fetuin-A with phosphorus levels (r=-0.819; p<0.01). Serum calcium levels were found to be significantly reduced in CKD cases [Mean level: Cases- 9.54±0.67mg/dl; Controls- 9.9±0.41mg/dl; p=0.001]. Fetuin-A showed a significant positive correlation with serum calcium (r=0.464; p<0.01).

Similar to serum phosphorus, our results also showed a highly significant increase of calcium-phosphorus product (Ca X P) in CKD cases [Mean level: Cases- 42.84±8.7mg^2/dl^2; Controls- 33.71±3.28mg^2/dl^2; p=0.001] and a progressive increase in Ca X P as the renal function declined. We also found a strong significant inverse correlation of serum Fetuin-A with Ca X P (r=-0.818; p<0.01). Similar findings were observed in the previous studies[84,85].

The possible mechanism for this observation could be that, serum Fetuin-A is a circulating inhibitor of vascular and soft tissue calcification and it avidly binds to both calcium and phosphate in the serum forming small calciprotein particles which are removed by macrophages. Fetuin-A thus acts as a "buffer" of serum calcium-phosphate to prevent extraskeletal calcification. In CKD, increased serum levels of calcium-phosphate may consume the circulating Fetuin-A and thereby decrease its levels.

Dyslipidemia, an atherosclerotic risk factor, contributes to the initiation and progression of CKD partly by stimulating and amplifying the effect of inflammatory mechanisms[87]. In the present study, we observed a significantly higher serum TGL and VLDL-C levels in cases than controls ($p=0.001$). Serum TC and LDL-C were found to be within the normal reference range in our study group. Further we observed a significant negative correlation of serum Fetuin-A with TGL and VLDL-C ($r=-0.366$; $p<0.01$) and a positive correlation of serum Fetuin-A with HDL-C ($r=0.443$; $p<0.01$).

These findings are in accordance with the study of Zeiden et al., who in addition observed elevated levels of TC and LDL-C[86]. The reason for this conflicting result could be explained by the fact that, in CKD patients with concomitant inflammation and malnutrition, TC and LDL-C levels may be low.

Previous studies have demonstrated that reduced serum Fetuin-A levels could be considered as a predictor of both cardiovascular and non-cardio vascular mortality[88,92]. Hermans et al., found that an increment of 0.1g/L concentration of serum Fetuin-A resulted in a 13% reduction in the all-cause mortality[6]. Cagler et al., demonstrated that short term treatment with Sevelamer, a non-calcium-based phosphate binder in patients with CKD, increased serum Fetuin-A concentration which in turn improved the endothelial dysfunction in these patients[93].

Taken together, the results of the present study suggest that since Fetuin-A is a circulating inhibitor of calcium-phosphate precipitation, the available Fetuin-A is depleted in dealing with the elevated Ca X P commonly found in CKD. Further, in the chronic inflammatory state of CKD, the synthesis of Fetuin-A, a negative acute phase reactant is down regulated. Hence, prolonged exposure to a pro-calcific and a pro-inflammatory environment in CKD eventually lead onto the reduced production and increased consumption of serum Fetuin-A. These findings highlight the close relationship between inflammation and vascular calcification in CKD.

CONCLUSION

- The present study demonstrated that serum Fetuin-A concentrations are significantly reduced in patients with CKD. This decrease in Fetuin-A levels is progressive, beginning from the early stages of CKD.
- The chronic inflammatory state and the altered mineral metabolism prevailing in CKD could be responsible for the lowered levels of serum Fetuin-A.
- Thus, vascular calcification and the resultant cardiovascular disease are likely to develop early during the progression of CKD.
- Since no approved measures are available currently to increase the serum Fetuin-A levels, early and aggressive treatment of inflammation, hyperphosphatemia and dyslipidemia may favour an increase in the serum Fetuin-A concentrations and subsequently slow down the accelerated course of cardiovascular disease in CKD.

LIMITATIONS OF THE STUDY

- Application of imaging techniques would have helped us to evaluate the extent of vascular calcification in CKD.
- Studies on Fetuin-A gene polymorphism would have helped us to evaluate the impact of variations in the gene encoding Fetuin-A on the protein product and outcome.

SCOPE FOR FURTHER STUDY

With cardiovascular disease being increasingly viewed to be mediated partly by inflammatory processes, Fetuin-A which is a negative acute phase reactant, might prove to be a valuable therapeutic target to prevent vascular calcification and mortality in CKD. Strategies aimed at enhancing the serum levels of Fetuin-A may have therapeutic benefits.

ANNEXURES

BIBLIOGRAPHY

1. Martin E Lascano, Martin J Schreiber and Saul Nurko. Chronic Kidney Disease. In: William D. Carey, editor. Cleveland Clinic Current Clinical Medicine. 2nd Edition. Philadelphia: Elsevier Saunders; 2010. p. 853
2. Andrew S Levey, Kai-Uwe Eckardt, Yusuke Tsukamoto et al. Definition and Classification of Chronic Kidney Disease: Improving Global Outcomes (KDIGO). Kidney International. 2005; Vol.67: 2089-2100
3. Cozzolino M, Brancaccio D, Gallieni M et al. Pathogenesis of Vascular Calcification in Chronic Kidney Disease. Kidney Int. 2005; Vol. 68: 429-436
4. Juan F Navarro-Gonzalez, Carmen Mora-Fernandez, Mercedes Muros et al. Mineral Metabolism and Inflammation in Chronic Kidney Disease Patients: A Cross Sectional Study. Clin J Am Soc Nephrol. 2009; 4: 1646-1654
5. Schiftrin EL, Lipman ML and Johannes FE Mann. Cardiovascular Involvement in General Medical Conditions, Chronic Kidney Disease-Effects on the CVS. Circulation. 2007;116: 85-97
6. MMH Hermans, V Brandenburg and M Ketteler et al. Association of Serum Fetuin-A Levels with Mortality In Dialysis Patients. Kidney International. 2007; 72: 202-207

7. Sharon M Moe and Neal X Chen. Pathophysiology of Vascular Calcification in Chronic Kidney Disease. Circulation Research. 2004; 95: 560-567
8. Martin A Crook. The kidneys. In: Martin A Crook, editor. Clinical Chemistry and Metabolic Medicine. 7th edition. London: Edward Arnold (publishers) Ltd; Book Power edition, 2006. p. 37-38
9. Sui Phin Kon and William J Marshall. The Kidneys, Renal Function and Renal Failure. In: William J Marshall and Stephen K Bangert, editors. Clinical Biochemistry, Metabolic and Clinical aspects. 2nd Edition. Edinburgh: Churchill Livingstone Elsevier; 2008. p. 131-132
10. Widmaier EP, Raff, Strang KT. The kidneys and regulation of water and inorganic ions. In: Wheatley CH, editor. Vander's Human Physiology, The Mechanisms of Body Function. 11th Edition. New York: McGraw-Hill; 2006. p. 492-496
11. George A Tanner. Kidney function. In: Rodney A Rhoades and David R Bell, editors. Medical Physiology, Principles for Clinical Medicine. 3rd Edition. Baltimore: Wolters Kluwer, Lippincott Williams & Wilkins; 2009. p. 401-403
12. Susan E Mulroney and Adam K Myers. Renal transport processes. In: Grady EO, editor. Netter's Essential Physiology. 1st edition. Philadelphia: Saunders Elsevier Publications; 2009.p. 209

13. Lauralee Sherwood. The Urinary system. In: Sherwood L, editor. Human Physiology from Cells to Systems. 4th edition. California: Brookes/Cole Thomson Learning; 2009. p. 496
14. Leonard V Crowley. The Urinary system. In: An introduction to human disease – pathology and pathophysiology correlations. 8th edition. Massachusetts: Jones and Bartlett publishers; 2010. p. 485
15. National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification. Am J Kidney Disease. 2002; 39: Suppl 1: S1-S266
16. Michael P Delaney, Christopher P Price, David J Newman and Edmund Lamb. Kidney Diseases. In: Carl A Burtis, Edward R Ashwood and David E Burns, editors. Tietz Text Book of Clinical Chemistry and Molecular Diagnostics. 4th edition. Missouri: Saunders Elsevier; 2006. p.1690
17. Joshua M Kaplan and Martin Roy First. Renal Function. In: Lawrence A Kaplan and Amadeo J Pesce, editors. Clinical Chemistry- Theory, Analysis, Correlation. 5th Edition. Missouri: Mosby Elsevier; 2010. p. 578
18. Kunihiro M, Elizabeth S, Lori DB et al. RISK Implications of the New CKD Epidemiology Collaboration (CKD-EPI) Equation Compared With the MDRD Study Equation for Estimated GFR: The Atherosclerosis Risk In Communities (ARIC) Study. American Journal of Kidney Diseases. 2010; Vol. 55, No.4 (April): 648-659

19. Prabahar MR, Venkatraman C and Soundararajan P. Epidemic Of Chronic Kidney Disease In India- What Can Be Done? Saudi J Kidney Dis Transpl. 2008; 19(5):847-853
20. Sanjay K Agarwal. Chronic Kidney Disease and Its Prevention in India. Kidney International. 2005; Vol. 68, Supplement 98: S41-S45
21. Stenvinkel P. Chronic Kidney Disease: A Public Health Priority and Harbinger of Premature Cardiovascular Disease. J Intern Med. 2010; 268: 456–467
22. Madhumathi Rao and Brian JG Pereira. Chronic Kidney Disease in India- A Hidden Epidemic. Indian J Med Res. 2007 July; 126: 6-9
23. Franch HA, McCellan WC, Mitch WE. Chronic Kidney Disease: Patho physiology And Influence of Dietary Protein, In: Robert J Alpern and Steven C Hebert, editors. Seldin and Giebisch's The Kidney. Vol.2. 4th edition. California: Elsevier Publications; 2008. p. 2624-2625
24. Koss MEL and Nahas MEL, Epidemiology and Pathophysiology of Chronic Kidney Disease: Natural History, Risk Factors and Management; Wheeler DC and Winearls CG, Clinical evaluation and management of chronic kidney disease; In: Feehally J, Floege J and Johnson RJ, editors. Comprehensive Clinical Nephrology. 3rd Edition. Philadelphia: Mosby Elsevier; 2007. p. 814,832
25. Lesley A Stevens, Nicholas Stoychaff and Andrew S Levey. Staging and Management of Chronic Kidney Disease. In: Arthur Greenberg, editor.

Primer on Kidney Diseases. 5th Edition. Philadelphia: Saunders Elsevier; 2009. p. 436-437

26. Roderick PJ and Feest T. The epidemiology of renal disease. In: Davidson AM, Cameron JS, Grunfeld JP et al., editors. Oxford Textbook of Clinical Nephrology. Volume 1. 3rd Edition. New York: Oxford University Press; 2005. p. 202-206
27. MM Yaqoob. Renal disease. In: Parveen Kumar and Michael Clark, editors. Kumar and Clark's Clinical Medicine.7th edition. Spain: Saunders Elsevier; 2009. p. 625
28. Gerald A Hladik. Chronic Kidney Disease. In: Marshall S Runge and M Andrew Greganti, editors. Netter's Internal Medicine. 2nd edition. Philadelphia: Saunders Elsevier; 2009. p. 979
29. Sue E Huether and Mikel Gray. Alterations of Renal and Urinary Tract Function. In: Lee-Ellen C Copstead and Jacquelyn L Banasik, editors. Understanding Pathophysiology. 4th Edition. Missouri: Mosby Elsevier; 2008. p. 802
30. Roberta J Emerson. Acute Renal Failure and Chronic Kidney Disease. In: Lee-Ellen C Copstead and Jacquelyn L Banasik, editors. Pathophysiology. 4th Edition. Missouri: Saunders Elsevier; 2010. p. 691-692
31. Willian F Keane and Andrews S Levey. Effects of Diet and Lipid-Lowering Agents on the Progression of Renal Disease, In: Jacobson HR, Striker GE and Klahr S, editors. The Principles and Practice of

Nephrology. 2nd Edition. Missouri: Mosby Publications; 1995. p. 644-646

32. Carol Mattson Porth. Renal failure. In: Zuccarini, editor. Pathophysiology- Concepts of Altered Health States. 7th Edition. Philadelphia: Lippincott William Wilkins; 2005. p. 839
33. Stenvinkel P, Carrero JJ, Axelsson J et al. Emerging Biomarkers For Evaluating Cardiovascular Risk In The Chronic Kidney Disease Patient: How Do New Pieces Fit Into The Uremic Puzzle? Clin J Am Soc Nephrol. 2008; 3: 505-521
34. Hanna Abboud and William L.Henrich. Stage IV Chronic Kidney Disease. The New England Journal of Medicine. 2010; 362: 56-65
35. Ketteler M, Schlieper G, Floege J, Calcification and Cardiovascular health - New insights into an old phenomenon. Hypertension. 2006; 47:1027-1034
36. Lawrence P McMahon and Patrick S Parfrey. Cardiovascular Aspects of Chronic Kidney Disease. In: Bary M. Brenner, editor. Brenner and Rector's The Kidney. Vol.2. 8th Edition. Philadelphia: Saunders Elsevier; 2008. p. 1697-1713
37. Paul Muntner, Josef Coresh, J Clinton Smith. Plasma Lipids and Risk of Developing Renal Dysfunction: The Atherosclerosis Risk in Communities Study. Kidney International. 2000; Vol. 58: 293–301
38. Weiner DE and Sarnak MJ. Cardiovascular Disease in Patients with Chronic Kidney Disease, In: Chronic Kidney Disease, Dialysis and

Transplantation – Companion To Brenner And Rector's The Kidney, edited by Brian JG Pereira, Sayegh MH, Peter G Blake, 2nd Edition, 2005, Elsevier Saunders, Page 162

39. GA Kaysen. Dyslipidemia in Chronic Kidney Disease: Causes and Consequences. Kidney International. 2006; 70: S55–S58.
40. Vasilis Tsimihodimos, Zoi Mitrogianni and Moses Elisaf. Dyslipidemia Associated With Chronic Kidney Disease. The Open Cardiovascular Medicine Journal. 2011; 5: 41-48
41. Andrew P Sage, Yin Tintut and Linda L Demer. Regulatory Mechanisms in Atherosclerotic Calcification. Nat Rev Cardiol. 2010 September; 7(9): 528-536
42. Georgi Abraham, Varun Sundaram, Vivek Sundaram. C-Reactive Protein, a Valuable Predictive Marker In Chronic Kidney Disease. Saudi J Kidney Dis Transpl. 2009; 20(5): 811-815
43. Joao Egidio Romao Jr., Adlei Rogerio Haiashi, Rosilene Mota Elias. Positive Acute-Phase Inflammatory Markers in Different Stages of Chronic Kidney Disease. Am J Nephrol. 2006;26:59-66
44. S Huybers and RJM. Bindels. Vascular Calcification in Chronic Kidney Disease. Kidney Int. 2007; 72: 663-665
45. Masahide Misobuchi, Dwight Towler, Eduardo Slakopolsky et al. Vascular Calcification: The Killer of Patients with Chronic Kidney Disease. J Am Soc Nephrol. 2009; 20:1453-1464

46. Keith A Hruska, Mathew S, Memon I et al. The Pathogenesis of Vascular Calcification in CKD-MBD. Semin Nephrol. 2009 March; 29(2): 156-165
47. Kosaku Nitta. Vascular Calcification in Patients with Chronic Kidney Disease. Therapeutic Apheresis and Dialysis. 2011; Vol.15 (6): 513-521
48. Reynolds JL, Joannides AJ, Skepper JN et al. Human Smooth Muscle Cells Undergo Vesicle-Mediated Calcification in Response to Changes in Extracellular Calcium and Phosphorus Concentrations: A Potential Mechanism For Accelerated Vascular Calcification in ESRD. J Am Soc Nephrol. 2004; 15: 2857-2867
49. Sharon M Moe And Neal X Chen. Mechanisms of Vascular Calcification in Chronic Kidney Disease. J Am Soc Nephrol. 2008; 19: 213-216
50. M Kettler and C Giachelli. Novel Insights into Vascular Calcification. Kidney Int. 2006; 70: S5-S9
51. A Connor. Novel Therapeutic Agents and Strategies for the Management of CKD-MBD. Postgrad Med J. 2009; 85: 274-279
52. Uhlig K, Berns JS, Bryan Kestenbaum et al. KDOQI US Commentary on the 2009 KDIGO Clinical Practice Guideline For The Diagnosis, Evaluation and Treatment of CKD-MBD. American Journal of Kidney Diseases. 2010 May; Vol. 55 (5): 773-799

53. Jordi Goldstein-Fuchs and Denis Fouque. The Ubiquitous Nature and Elusive Role of Phosphorus and Vascular Calcification. American Journal of Kidney Diseases. 2009; Vol. 53, No. 3 (March): 363-365
54. Dominique Prie and Gerard Friedlander. Genetic Disorders of Renal Phosphate Transport. N Eng J Med. 2010; 362: 2399-2400
55. Marcello Tonelli, Neesh Pannu, Braden Manns, Oral Phosphate Binders in Patients with Kidney Failure. N Eng J Med. 2010; 362; 14:1315
56. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W. The Serum Protein α_2-Heremans-Schmid Glycoprotein/Fetuin-A is a Systemically Acting Inhibitor of Ectopic Calcification. J Clin Invest. 2003; 112: 357–366
57. Sharon M Moe. Vascular calcification: The three- hit model. J Am Soc Nephrol. 2009; 20: 1162-1164
58. Andrzej Elzanowski, Winona C Barker, Lois T Hunt et al. Cystatin Domains in Alpha-2-HS-Glycoprotein and Fetuin. Federation of European Biochemical Societies. January 1988; Vol.227(No.2): 167-170
59. Yasuyuki Yoshika, Fumitake Gejyo, Thomas Marti et al. The Complete Aminoacid Sequence of the A-Chain of Human Plasma α_2 – HS Glycoprotein. The Journal of Biological Chemistry. 1986 February 5; Vol.261(No.4): 1665-1676
60. Josef Kellermann, Heinz Haupt, Ernst-August et al. The arrangement of disulfide loops in α_2 – HS glycoprotein, similarity to the disulfide bridge

structures of Cysatins and Kininogens. The Journal of Biological Chemistry. 1989 August 25. Vol.264 (No.24): 14121-14128

61. Chong-Chou Lee, Barbarah Bowman and Funmei Yang. Human α_2 – HS Glycoprotein: The A and B chains with a connecting sequence are encoded by a single mRNA transcript. Biochemistry. July 1987; Vol. 84: 4403-4407
62. Peter Nawratil, Sabine Lenzen, Josef Kellermann et al. Limited proteolysis of human α_2 – HS glycoprotein/Fetuin, evidence that chymotryptic activity can release the connecting peptide. The Journal of Biological Chemistry. December 6, 1996; Vol. 271 (No. 49): 31735-31741
63. Willi Jahnen-Dechent, Andeas Trindl et al. Posttranslational Processing of Human α_2 – HS Glycoprotein (Human Fetuin), evidence for the production of a phosphorylated single-chain form by hepatoma cells. European Journal of Biochemistry. 1994; 226: 59-69
64. Chunsik Lee, Erik Bongcam-Rudloff, Christian Sollner et al. Type 3 Cystatins; Fetuins, Kininogen and Histidine-Rich Glycoprotein. Frontiers in Bioscience. 2009, January 1; 14: 2911-2922
65. Rukshana C. Shroff, Vanita Shah, Melanie P Hiorns et al. The Circulating Calcification Inhibitors, Fetuin-A and Osteoprotegerin, But Not Matrix Gla Protein, are Associated with Vascular Stiffness and Calcification in Children on Dialysis. Nephrol Dial Transplant. 2008; 23: 3263-3271

66. Christoph Binkert, Michael Demetriou, Balram Sukhu et al. Regulation of Osteogenesis by Fetuin. The Journal of Biological Chemistry. October 1, 1999; Vol. 274 (No.40): 28514-28520
67. Martin Hausler, Cora Schafer, Claudia Osterwinter et al. The Physiologic Development of Fetuin-A serum Concentrations in Children. Pediatric Research. 2009; Vol. 66 (No.6): 660-664
68. Alexander Heiss, Alexander Duchesne, Bernd Denecke et al. Structural Basis of Calcification Inhibition by α_2 – HS Glycoprotein/Fetuin-A. Formation of Colloidal Calciprotein Particles, The Journal Of Biological Chemistry, 2003, April 11; Vol. 278 (No.15): 13333-13341
69. Joanne L Reynolds, Jeremy N Skepper, Rosamund Mcnair et al. Multifunctional Roles for Serum Protein Fetuin-A in Inhibition of Human Vascular Smooth Muscle Cell Calcification. J Am Soc Nephrol. 2005;16: 2920-2930
70. Jean Pierre Lebreton Francois Joisel, Jean Pirre Raoult and Bernard Lannuzel et al. Serum Concentration Of Human $Alpha_2$ – HS Glycoprotein During The Inflammatory Process, Evidence That $Alpha_2$ – HS Glycoprotein is a Negative Acute-Phase Reactant. J Clin Invest. October 1979; Vol. 64: 1118-1129
71. Gangneux C, Daveau M, Hiron M et al. The Inflammation-Induced Down-Regulation of Plasma Fetuin-A (α_2 – HS Glycoprotein) in Liver Results from the Loss of Interaction between Long C/EBP Isoforms at

Two Neighbouring Binding Sites. Nucleic Acids Research. 2003; Vol. 31 (No.20): 5957-5970

72. Paul A Price and Joo Eun Lim. The Inhibition of Calcium Phosphate Preciptitation by Fetuin is accompanied by the Formation of a Fetuin-Mineral Complex. The Journal of Biological Chemistry. June 13, 2003; Vol. 278 (No. 24): 22144-22152
73. H Wang, M Zhang, M Bianchi. Fetuin (α2-HS-Glycoprotein) Opsonizes Cationic Macrophage Deactivating Molecules (CNI-1493yp38 Mitogen-Activated Protein Kinase). Proc. Natl. Acad. Sci. USA. November 1998; Vol. 95: 14429–14434
74. Michael Demetriou, Christoph Binkert, Balram Sukhu et al. Fetuin/ α_2 – HS Glycoprotein is a Transforming Growth Factor-B Type II Receptor Mimic and Cytokine Antagonist. The Journal of Biological Chemistry. 1996, May 31; Vol. 271(No. 22): 12755- 12761
75. Cagler K, Yilmaz MI, Saglam M et al. Serum Fetuin-A Concentration and Endothelial Dysfunction in CKD. Nephron Clin Pract. 2008; 108: C233-C240
76. Kettler M, Bongartz P, Westenfeld R. Association of Low Fetuin-A (AHSG) Concentrations in Serum with Cardiovascular Mortality in Patients on Dialysis: A Cross-Sectional Study. Lancet. 2003; 361: 827-833

77. Oikawa O, Higuchi T, Yamazaki T. Evaluation of Serum Fetuin-A Relationships with Biochemical Parameters in Patients on Hemodialysis. Clin Exp Nephrol. 2007 Dec; 11(4): 304-8
78. Santina Cottone, Alessandro Palermo, Rosalia Arsena et al. Relationship of Fetuin-A with Glomerular Filtration Rate and Endothelial Dysfunction in Moderate-Severe Chronic Kidney Disease. J Nephrol. 2010; 23 (01): 62-69
79. Nadia A Hussein, Ola M Mahmoud, Manal Y Zahran et al. Serum Fetuin-A in Chronic Renal Disease Patients: Contribution to Endothelial Dysfunction and Hemostatic Alteration. Journal of American Science. 2010; 6 (12): 1098-1102
80. Stenvinkel P, Wang K Qureshi Ar et al. Low Fetuin-A Levels are Associated with Cardio Vascular Death: Impact of Variations in the Gene Encoding Fetuin. Kidney Int. 2005 Dec; 68(6): 2915
81. Joane M Bargman and Karl S Korecki. Chronic Kidney Disease. In: Longo DL, Kasper DL, Jameson JL et al., editors. Harrison's Principle of Internal Medicine. Vol.2. 18th Edition. New York: McGraw Hill; 2012. p. 2310
82. Hirokazu Honda, Abdul Rashid Qureshi, Olof Heimburger et al. Serum Albumin, C-Reactive Protein, Interleukin 6, and Fetuin-A as Predictors of Malnutrition, Cardiovascular Disease, and Mortality in Patients with ESRD. American Journal of Kidney Diseases. January 2006; Vol. 47(No. 1): 139-148

83. CP Kovesdy, JE Anderson and K Kalantar-Zadeh. Outcomes Associated with Serum Phosphorus Level in Males With Non-Dialysis Dependent Chronic Kidney Disease. Clinical Nephrology. 2010; Vol.73 (No.4): 268-275
84. V Leziac, B Tirmenstajn-Jankovic, D Bukvic et al. Efficacy of Hyper phosphatemia Control in the Progression of Chronic Renal Failure and the Prevalence of Cardiovascular Calcification. Clinical Nephrology. 2009; Vol.71 (No.1): 21-29
85. Giovanni Pertosa, Simona Simone, Marco Ciccone et al. Serum Fetuin-A in Hemodialysis: A Link Between Derangement of Calcium-Phosphorus Homeostasis and Progression of Atherosclerosis? American Journal of Kidney Diseases. March 2009; Vol.53 (No.3): 467-474
86. Mohammed A Zeidan, Gihan M Sharara, Howaidans Suliman et al. Visfatin and Fetuin-A: Novel Markers for Endothelial Dysfunction in Chronic Kidney Disease. Life Science Journal. 2012; 9(2): 227-239
87. Linda F Fried, Trevor J Orchard and Bertram L Kasiske. Effect of Lipid Reduction on the Progression of Renal Disease: A Meta-Analysis Kidney International. 2001; Vol.59: 260–269
88. Sharon M Moe, Martina Reslerova, Markus Ketteler et al. Role of Calcification Inhibitors in the Pathogenesis of Vascular Calcification in Chronic Kidney Disease (CKD). Kidney International. 2005;Vol.67: 2295-2304

89. R Mehrotra, Emerging Role for Fetuin-A as Contributor to Morbidity and Mortality in Chronic Kidney Disease. Kidney International. 2007; 72: 137-140
90. Hung-Yuan Chen, Yen-Ling Chiu, Yi-Fang Chuang et al. Association of Low Serum Fetuin-A Levels with Poor Arteriovenous Access Patency in Patients Undergoing Maintenance Hemodialysis. American Journal of Kidney Diseases. October 2010; Vol. 56 (No.4): 720-727
91. Wang AY, Woo J, Lam CW et al. Associations of Serum Fetuin-A with Malnutrition, Inflammation, Atherosclerosis and Valvular Calcification Syndrome and Outcome in Peritoneal Dialysis Patients. Nephrol Dial Transplant. 2005 Aug; 20(8): 1676-1685
92. HH Jung, HJ Baek and SW Kim. Fetuin-A, Coronary Artery Calcification and Outcome in Maintenance Hemodialysis Patients. Clinical Nephrology. 2011; Vol.75 (No.5): 391-396
93. Kayser Cagler, Mahmut Ilker Yilmaz, Mutlu Saglam et al. Short Term Treatment with Sevelamer Increases Serum Fetuin-A Concentration and Improves Endothelial Dysfunction in Chronic Kidney Disease Stage 4 Patients. Clin J Am Soc Nephrol. January 2008; Vol.3(No.1): 61-68

A STUDY OF SERUM FETUIN-A LEVEL IN PATIENTS WITH CHRONIC KIDNEY DISEASE

PROFORMA

Name of the patient : OP/IP No.:

Age/sex :

Occupation :

Address :

Complaints : Oliguria / nocturia/ dysuria/ pedal edema/ anasarca/ fever/ weight loss

Past history : Hypertension/ Diabetes/ Tuberculosis/ Cerebrovascular disease/ Liver disease/ Rheumatoid arthritis

Personal history : Diet/ Tobacco/ alcohol/ cigarette use

Family history : Hypertension/ Diabetes/ Renal disease

Treatment history : Drugs (lipid lowering drugs, calcium/phosphate binders)/ Renal replacement therapy (Hemodialysis/ Peritoneal dialysis/ renal transplantation)

General examination:

Height: cms; Weight: kg; BMI: kg/m^2

Pulse Rate: /min; Blood Pressure: mmHg

Systemic examination:

Cardiovascular system:

Respiratory system:

Abdomen:

Central nervous system:

PROVISIONAL DIAGNOSIS:

INVESTIGATIONS:

1) Blood Glucose (Fasting) : mg/dl
2) Blood Urea : mg/dl
3) Serum Creatinine : mg/dl
4) Creatinine Clearance : ml/min.
5) Serum Fetuin-A : g/L
6) Serum hsCRP : mg/L
7) Serum Calcium : mg/dl
8) Serum Phosphorus : mg/dl
9) Ca X P : mg^2/dl^2
10) Serum Albumin : g/dl
11) Serum Lipid Profile:
    - Total Cholesterol : mg/dl
    - Triglycerides : mg/dl
    - HDL-C : mg/dl
    - LDL-C : mg/dl
    - VLDL-C : mg/dl

CONSENT FORM

Dr.P.Deepa, postgraduate student in the department of Biochemistry, Thanjavur Medical College, Thanjavur is doing a study on serum Fetuin-A level in patients with chronic kidney disease. The procedures have been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in the study. The data obtained here may be used for research and publication.

Signature:

Name:

Place: